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Regulation of Free Radical Processes by Delta-Sleep Inducing Peptide in Rat Tissues under Cold Stress

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Abstract—An intraperitoneal injection of an exogenous delta-sleep inducing peptide (DSIP) at a dose of 12 μg/100 g body weight shifted the prooxidant–antioxidant balance of free radical process (FRP) in tissues and erythrocytes of rats: the activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) and the concentrations of antioxidants (reduced glutathione in particular) increased. The DSIP stimulated the myeloperoxidase activity in blood neutrophils and had no effect on the activity of xanthine oxidase, a prooxidant enzyme, in the brain and liver. Cold stress displaced the prooxidant–antioxidant balance by increasing the xanthine oxidase activity in tissues and decreasing the myeloperoxidase activity in blood neutrophils; it also inhibited the enzyme antioxidant activities in tissues and erythrocytes that was neutralized by an increased ceruloplasmin activity in blood plasma and by an elevated level of antioxidants in rat blood and tissues. Preliminary administration of DSIP to animals exposed to cold stress restored the prooxidant–antioxidant balance: it normalized the myeloperoxidase activity in blood neutrophils, decreased the xanthine oxidase activity, and increased the activity of antioxidant enzymes in tissues and erythrocytes restoring the antioxidant level. The molecular regulation mechanism of free radical processes by DSIP in tissues under stressful conditions is discussed.

Key words: delta-sleep inducing peptide, stress, free radical processes, prooxidants, antioxidants, brain, liver, blood

Free radical oxidation plays a key role in the development of stressful and pathological conditions. Elevated concentrations of reactive oxygen species (ROS) caused by enhanced production or by exhaustion of the antioxidant system (AOS) activate destructive processes in the cells called "oxidative stress" [1, 2].

In a number of reviews, causes and mechanisms of prooxidant–antioxidant disbalance leading to oxidative stress are discussed; however, research in this area is mainly focused on the cytotoxic and destructive effects of highly reactive oxygen species, while the problems of their origin, initiation mechanisms, primary localization, and the processes involved in the prooxidant–antioxidant disbalance, as well as the regulatory aspects, remain unclear. The mode of action of delta-sleep inducing peptide (DSIP) and its role in the regulation of free radical oxidation are of particular interest. DSIP is an endogenous nonapeptide of 848.98 daltons with amino acid sequence Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu. The spatial structures of DSIP and its physiologically active analogs ([Ser^{7}HPO$_3$]DSIP, cyclo-(Gly-DSIP), Tyr-DSIP, and some others) have been determined. Folded quasi-cyclic forms are characteristic of DSIP [3-5].

The anti-stress and adaptive effects of DSIP, which was first identified as a hypnotic brain modulator [3] during emotional and pain reactions, cold stress, hyper- and hypoxia, and hypokinesis, are being studied intensively [6-10]. Since DSIP was found not only in the central nervous system (CNS), but also in all peripheral organs, tissues, and liquids of different animals, it was suggested to play a regulatory role [4]. The effect of DSIP on several functions simultaneously and its prolonged regulatory effect have been shown, being more pronounced under extreme conditions [11-13]. The objective of this work was to study the molecular mechanisms underlying the development of oxidative stress in tissues of rats under cold stress. We measured the activities of prooxidant and antioxidant enzymes, determined the concentrations of antioxidants responsible for the antioxidant status of the organism, and studied DSIP-mediated regulation of the prooxidant–antioxidant balance.

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MATERIALS AND METHODS

White, mature, non-pedigree male rats (150-180 g body weight) were studied in winter months. Control and experimental animals were subdivided into four groups. Group 1 animals kept in the vivarium at 18-20°C were used as a control. To group 2 animals, three days before decapitation, DSIP dissolved in sterile physiological solution at a dose of 12 μg per 100 g body weight was injected intraperitoneally (control + DSIP). Group 3 animals were kept in a refrigerating chamber at 0-4°C for three days (three days cold). According to stress and adaptation tests, the maintenance of animals in the refrigerating chamber for 1-3 days is considered stressful [14-17]. To group 4 animals, DSPI was administered similarly as described for group 2, whereupon they were kept in the refrigerating chamber for three days (three days cold + DSIP). DSIP penetrates through the blood–brain barrier during peripheral administration [18, 19]. The DSIP used in this work was synthesized in the Laboratory of Peptide Chemistry at the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (Russian Academy of Sciences, Moscow). In all experiments, DSIP was injected in the morning hours since the endogenous DSIP level has been shown to change during the day and in different seasons producing small effects in the organism that can finally lead to dramatic metabolic changes [20]. DSIP at a dose of 12 μg per 100 g body weight produced an antistress effect on animals exposed to low temperatures [21]. To avoid effects of metabolic fluctuations during the day, animals of all groups were decapitated in the morning hours three days after the beginning of the experiment; blood was collected in heparin-treated centrifuge tubes; the brain and liver were immediately placed in ice-cold physiological solution. Plasma was obtained from blood by centrifugation at 1,500*g* for 15 min. Erythrocytes were washed three times with cold physiological solution. Lymphocytes and neutrophils were isolated from blood in a Ficoll–verograffin density gradient according to Boyum [22]. Erythrocytes, neutrophils, blood plasma, homogenates, and supernatants of 20% liver and brain homogenates (w/v) in physiological solution treated with Triton X-100 (final concentration 0.1%) were used for analysis. Lipids were extracted from the liver and brain homogenates and erythrocyte membranes prepared from 1% hemolysates following the procedure of Bligh and Dyer [23]. Cholesterol was determined in chloroform extract as described by Ilk [24]; total lipids were measured by a sulfophosphovanilline procedure using the commercial Bio-La-Test TL 180 kit (Lachema, Czech Republic). Xanthine oxidase activity was determined according to Avis [25]. Myeloperoxidase was assayed spectrophotometrically according to Saidov and Pinegin [26] in a modification of Shustanova [27]. Protein concentration was measured according to Lowry [28]. The superoxide dismutase activity was determined by Fried's procedure [29]; catalase was measured according to Korolyuk et al. [30]. Glutathione peroxidase activity was assayed by the rate of reduced glutathione oxidation in the presence of tertiary butyl hydroperoxide by the method of Moin [31]. Glutathione reductase was measured by the rate of $NADPH_2$ oxidation as described by Yusupova [32]; the reduced glutathione concentration was determined according to Ellman [33] in a modification of Kornienko [34]. Hemoglobin concentration was estimated by a modified procedure of Karakashov and Vichev [35] using a Reagent kit (Russia). Ceruloplasmin oxidase activity was assayed by a modification of Revin's procedure [24]. The amount of medium-sized molecules (MSM) was determined by a screening procedure described by Gabrielyan and Lipatova [36]. Urea was determined by a standard diacetyl monooxime procedure using the commercial Bio-La-Test Urea 450 kit (Lachema); uric acid was measured by a colorimetric method using Folin–Denis phosphotungstic reagent with kit (Reagent, Russia).

The statistical significance of differences between the control and experimental groups were estimated by Student's *t*-test.

RESULTS AND DISCUSSION

Our data showed that a single intraperitoneal injection of DSIP at a concentration of 12 μg per 100 g body weight produced a significant effect on free radical processes (Tables 1-5).

We demonstrated that one of the mechanisms of action of DSIP involved activation of a phagocytic chain in the immune response manifested by an increase in the myeloperoxidase activity in blood neutrophils by 39% ($p < 0.01$) as compared to control. The injection of DSIP to control animals did not lead to significant changes in the activity of xanthine oxidase, a prooxidant enzyme, in the brain and liver.

The antioxidant enzymes, superoxide dismutase (SOD) and catalase, participate in the primary response of cells to reactive oxygen species. In rats injected with DSIP, an increase in SOD activity by 56% ($p < 0.001$), 61.6% ($p < 0.01$), and 40.2% ($p < 0.01$) was recorded in the brain, liver, and erythrocytes, respectively. The catalase activity increased in these tissues by 38% ($p < 0.001$), 43.3% ($p < 0.001$), and 40.5% ($p < 0.001$), respectively. The next mechanism of cell defense from oxidative stress involves glutathione peroxidase. DSIP induced activation of glutathione peroxidase and glutathione reductase by 37% ($p < 0.01$) and 50% ($p < 0.001$) in the brain, by 48.7% ($p < 0.001$) and 28% ($p < 0.001$) in the liver, and by 15% ($p < 0.001$) and 42.4% ($p < 0.01$), respectively, in the erythrocytes. The concentration of reduced glutathione, a nonenzymatic component of the glutathione peroxidase system, increased even more in the experimental animals: by 90.5% ($p < 0.001$), 51.6% ($p < 0.001$), and 28.4% ($p <$

Table 1. Xanthine oxidase activity (mU/mg protein) in the brain and liver and myeloperoxidase activity (relative extinction units/mg protein per min) in blood neutrophils after cold treatment and DSIP injection ($M \pm m$, $n = 8$-18)

Parameter	Control	Control + DSIP	Three days cold	Three days cold + DSIP
		Brain		
Xanthine oxidase	1.18 ± 0.05	1.30 ± 0.06	2.19 ± 0.10*	1.54 ± 0.06***
		Liver		
Xanthine oxidase	1.74 ± 0.10	1.58 ± 0.05	3.37 ± 0.08*	2.38 ± 0.12***
		Blood neutrophils		
Myeloperoxidase	0.063 ± 0.004	0.088 ± 0.006*	0.042 ± 0.004*	0.062 ± 0.004**

* Significant differences from control ($p < 0.01$-0.001).
** Significant differences from three days cold ($p_1 < 0.01$-0.001).

0.001) in the brain, liver, and erythrocytes, respectively. Increased glutathione concentrations were probably due to the activation of glutathione reductase. A considerable increase in the activity of the glutathione peroxidase system in tissues, especially in the brain, induced by DSIP injection plays an important role in the antioxidant (AO) defense. The administration of exogenous DSIP to control animals did not cause any changes in the oxidase activity of ceruloplasmin, an important antioxidant of blood plasma and one of the natural defense agents.

The DSIP-induced increase in AOS enzyme activities recorded in the brain, liver, and erythrocytes may result from activation of their synthesis either directly through transcription activation of the genes encoding AO enzymes or indirectly via hormones. A direct effect of DSIP on the activity of AOS enzymes cannot be excluded.

Medium-sized molecules (MSM) deserve special attention due to their diverse biological functions in the organism including the AO activity. DSIP injected to the

Table 2. Superoxide dismutase activity (relative units/mg protein or relative units/mg Hb) and catalase (U/mg protein or U/mg Hb) in the brain, liver, and erythrocytes of rats after cold treatment and DSIP injection ($M \pm m$, $n = 9$-18)

Parameter	Control	Control + DSIP	Three days cold	Three days cold + DSIP
		Brain		
Superoxide dismutase	14.36 ± 0.85	22.38 ± 1.93*	9.99 ± 1.05*	14.66 ± 0.96**
Catalase	4.18 ± 0.19	5.76 ± 0.22*	2.10 ± 0.17*	4.02 ± 0.13**
		Liver		
Superoxide dismutase	69.70 ± 9.83	112.65 ± 6.88*	32.34 ± 3.68*	75.02 ± 4.76**
Catalase	228.58 ± 9.60	327.59 ± 9.53*	113.63 ± 8.73*	251.9 ± 12.11**
		Erythrocytes		
Superoxide dismutase	21.24 ± 1.05	29.79 ± 2.09*	9.41 ± 0.42*	19.21 ± 1.11**
Catalase	112.63 ± 5.24	158.29 ± 7.16*	92.16 ± 3.99*	115.52 ± 7.13**

* Significant differences from control ($p < 0.01$-0.001).
** Significant differences from three days cold ($p_1 < 0.05$-0.001).

Table 3. Glutathione peroxidase activity (mU/mg protein or mU/mg Hb), glutathione reductase activity (mU/mg protein or mU/mg Hb), and the concentration of reduced glutathione (μmole/g tissue or μmole/g Hb) in the brain, liver, and erythrocytes of rats after cold treatment and DSIP injection ($M \pm m$, n = 8-18)

Parameter	Control	Control + DSIP	Three days cold	Three days cold + DSIP
	Brain			
Glutathione peroxidase	28.54 ± 2.15	39.10 ± 2.51*	17.41 ± 1.31*	33.61 ± 3.96**
Glutathione reductase	5.00 ± 0.13	7.49 ± 0.44*	3.85 ± 0.25*	5.31 ± 0.33**
Reduced glutathione	0.55 ± 0.03	1.04 ± 0.11*	0.59 ± 0.02	0.69 ± 0.03**
	Liver			
Glutathione peroxidase	232.50 ± 16.64	345.78 ± 8.19*	133.42 ± 5.21*	222.7 ± 29.68**
Glutathione reductase	12.71 ± 0.46	16.27 ± 0.55*	9.55 ± 0.34*	15.66 ± 0.76**
Reduced glutathione	2.58 ± 0.26	3.90 ± 0.16*	1.24 ± 0.08*	2.52 ± 0.24**
	Erythrocytes			
Glutathione peroxidase	541.45 ± 6.17	622.20 ± 3.70*	446.52 ± 6.89*	580.80 ± 21.33**
Glutathione reductase	5.90 ± 0.27	8.40 ± 0.68*	3.69 ± 0.35*	5.78 ± 0.46**
Reduced glutathione	7.69 ± 0.19	9.87 ± 0.47*	6.30 ± 0.20*	8.19 ± 0.40**

* Significant differences from control (p < 0.01-0.001).
** Significant differences from three days cold (p_1 < 0.05-0.001).

Table 4. Ceruloplasmin oxidase activity (μmole/liter) in blood plasma and the concentration of medium-sized molecules (relative extinction units) in blood plasma, liver, and brain after cold treatment and DSIP injection ($M \pm m$, n = 8-20)

Parameter	Control	Control + DSIP	Three days cold	Three days cold + DSIP
	Blood plasma			
Ceruloplasmin	1.60 ± 0.08	1.52 ± 0.07	2.68 ± 0.17*	1.91 ± 0.10**
E 254 nm	0.122 ± 0.007	0.130 ± 0.012	0.258 ± 0.006*	0.128 ± 0.003**
E 280 nm	0.186 ± 0.008	0.193 ± 0.013	0.301 ± 0.008*	0.206 ± 0.005**
$k = E_{280\ nm}/E_{254\ nm}$	1.56 ± 0.07	1.63 ± 0.14	1.17 ± 0.02 *	1.61 ± 0.04**
	Liver			
E 254 nm	0.788 ± 0.023	0.764 ± 0.019	0.586 ± 0.015*	0.763 ± 0.040**
E 280 nm	0.339 ± 0.013	0.336 ± 0.013	0.303 ± 0.018	0.354 ± 0.014**
$k = E_{280\ nm}/E_{254\ nm}$	0.43 ± 0.01	0.44 ± 0.02	0.52 ± 0.03*	0.47 ± 0.02
	Brain			
E 254 nm	0.781 ± 0.010	0.787 ± 0.015	0.658 ± 0.013*	0.850 ± 0.013**
E 280 nm	0.190 ± 0.008	0.185 ± 0.009	0.139 ± 0.004*	0.169 ± 0.004**
$k = E_{280\ nm}/E_{254\ nm}$	0.24 ± 0.01	0.23 ± 0.01	0.21 ± 0.01*	0.20 ± 0.01**

* Significant differences from control (p < 0.05-0.001).
** Significant differences from three days cold (p_1 < 0.05-0.001).

Table 5. Concentrations of urea (μmole/g tissue or mmole/liter) and uric acid (μmole/g tissue or μmole/liter) in the brain, liver, and blood plasma and the cholesterol concentration (μg/mg lipid) in the brain, liver, and erythrocytes of rats after cold treatment and DSIP injection ($M \pm m$, n = 8-22)

Parameter	Control	Control + DSIP	Three days cold	Three days cold + DSIP
	Brain			
Urea	3.74 ± 0.12	6.00 ± 0.21*	7.24 ± 0.38*	4.14 ± 0.55**
Uric acid	0.90 ± 0.05	1.42 ± 0.16*	1.77 ± 0.07*	1.30 ± 0.10**
Cholesterol	397.54 ± 17.31	319.17 ± 30.06*	477.16 ± 30.54*	345.98 ± 26.94**
	Liver			
Urea	5.70 ± 0.18	8.05 ± 0.44*	6.69 ± 0.21*	5.66 ± 0.28**
Uric acid	1.38 ± 0.05	1.86 ± 0.06*	1.35 ± 0.09	1.50 ± 0.09
Cholesterol	227.44 ± 19.86	219.11 ± 31.35	344.81 ± 32.31*	217.55 ± 44.87**
	Blood plasma/Erythrocytes			
Urea	5.36 ± 0.16	5.49 ± 0.16	7.28 ± 0.36*	5.54 ± 0.40**
Uric acid	85.71 ± 3.63	94.00 ± 5.46	169.70 ± 11.09*	113.33 ± 7.57***
Cholesterol	183.33 ± 16.67	141.46 ± 8.17*	249.03 ± 23.77*	151.59 ± 17.68**

* Significant differences from control ($p < 0.05$-0.001).
** Significant differences from three days cold ($p_1 < 0.05$-0.001).

control animals did not cause significant changes in the concentrations of peptide (prevailing in the blood plasma) and non-peptide (predominant in the liver and brain) MSM components as compared to control. The distribution index of these fractions $k = E_{280}/E_{254}$ in the samples examined was constant. The absence of differences in MSM concentrations in the blood plasma and tissues of control rats after DSIP injection probably indicates that, under these physiological conditions, catabolic processes in blood and/or tissues were not activated.

Two low-molecular-weight nitrogen-containing metabolites, urea and uric acid, play an important regulatory role and act as another mechanism of nonenzymatic AO defense. The injection of DSIP to animals increased the concentrations of urea and uric acid by 60.3% ($p < 0.001$) and 57.8% ($p < 0.01$), respectively, in the brain and by 41.2% ($p < 0.001$) and 35% ($p < 0.001$), respectively, in the liver. However, in the blood plasma these parameters were the same as in the control.

It is well known that, in addition to water-soluble low-molecular-weight AO, which exhibit their protective role in the cell cytoplasm and blood plasma, hydrophobic compounds are also important for the maintenance of AO homeostasis. They act through the inhibition of reactions proceeding in the membrane lipid layers and blood lipoproteins. After DSIP injection, the concentration of cholesterol, the key regulator of membrane fluidity, decreased by 19.7% ($p < 0.05$) and 22.8% ($p < 0.05$) in the brain and erythrocyte membranes, respectively, as compared to control animals. In the liver, no significant changes in cholesterol concentration were noted.

Thus, the protective role of DSIP consists in suppressing activation of lipid peroxidation (LPO) and in shifting the prooxidant–antioxidant balance towards higher AOS concentrations in tissues and blood, thus providing a guarantee against extreme LPO activation and preventing cell damage. Note that DSIP activates different enzymes involved in the AO defense and increases the concentration of the low-molecular-weight AO compounds in tissues and erythrocytes. This effect decreases in tissues as follows: brain, liver, and blood.

Before we studied the mechanism of the DSIP-induced anti-stress effect, we analyzed the above-mentioned biochemical parameters in tissues and blood under cold stress.

We recorded considerable changes in metabolic processes in animals exposed to low temperatures for three days, especially in the blood and liver. Smaller changes were observed in the brain (Tables 1-5).

Cold treatment was accompanied by a drastic increase in the xanthine oxidase activity in the brain and liver: by 86.5% ($p < 0.001$) and 93.4% ($p < 0.001$), respec-

tively. The myeloperoxidase activity decreased in the neutrophils of cold-treated animals by 34% ($p < 0.001$).

A disbalance in the LPO regulation system caused by the activation of initiation reactions is aggravated by a decreased AOS activity. Cold stress suppressed AOS by inhibiting AO enzymes, SOD and catalase, by 30.4% ($p < 0.01$) and 50% ($p < 0.001$) in the brain, by 53.6% ($p < 0.01$) and 50.3% ($p < 0.001$) in the liver, and by 55.7% ($p < 0.001$) and 18.2% ($p < 0.01$) in rat erythrocytes, respectively. Different rate of AO enzyme inhibition formed the basis for ROS accumulation. Cold stress changed the activity of glutathione-dependent enzymes. Thus, the activity of glutathione peroxidase and glutathione reductase decreased by 39% ($p < 0.001$) and 23% ($p < 0.001$) in the brain, by 42.6% ($p < 0.001$) and 24.8% ($p < 0.001$) in the liver, and by 17.5% ($p < 0.001$) and 37.4% ($p < 0.001$) in the erythrocytes of cold-treated rats in comparison with control animals. The reduced glutathione concentration in the experimental animals under cold stress reduced by 51.8% ($p < 0.001$) in the liver and by 18% ($p < 0.001$) in the erythrocytes; in the brain, it did not differ significantly from the control, which can be regarded as a specific defense mechanism maintaining a reserve level of SH-groups. Inactivation of AO enzymes in the brain, liver, and erythrocytes may be due to the inhibitory effect of ROS—$O_2^{\cdot}$ and $OH^{\cdot}$—accumulated as a result of xanthine oxidase activation in the studied tissues, which induces oxidative modification and conformational changes of enzyme molecules, and to their impaired synthesis or degradation. A complex of cytosolic macrooxyproteases located in erythrocytes and reticulocytes can recognize and selectively degrade oxidized AO proteins [37]. Decreased activity of AO enzymes in rat erythrocytes under cold stress may be related to dilution of the erythrocyte population with the "old" forms with low activity or to a release of erythrocyte enzymes into the blood plasma during hemolysis.

Under AOS inhibition in tissues, the specific proteins of blood plasma (especially ceruloplasmin) and nonenzymatic mechanisms of inhibition of free radical reactions become particularly important. At low temperatures, an increase in the ceruloplasmin activity by 67.3% ($p < 0.001$) was observed in the blood plasma.

Under cold stress, excess accumulation of the end products and intermediate metabolites in fluids and tissues of the organism was observed. This followed from an increased content of MSM in the blood plasma of the experimental animals: the concentration of a non-peptide fraction increased by 111% ($p < 0.001$) and that of a peptide fraction, by 62% ($p < 0.001$). A decrease in the k coefficient value (by 25.2%; $p < 0.001$) in the animals exposed to cold stress indicated that the MSM non-peptide component of the blood plasma was more sensitive to cold. The distribution of MSM fractions in tissues of animals under stress was different: the concentration of the non-peptide component in liver and brain decreased by 25.6% ($p < 0.001$) and 15.7% ($p < 0.001$), respectively; the concentration of the peptide component decreased by 26.6% ($p < 0.001$) in the brain; its concentration in the liver was not significantly affected by the stressful conditions; only slight changes were observed under stress. The k coefficient increased by 19.5% ($p < 0.05$) in the liver of cold-treated rats, pointing to larger changes in the non-peptide MSM fraction, and it decreased by 13% ($p < 0.01$) in the brain. This finding shows variation in MSM constituents, with a peptide component changing more considerably. Cold stress led to a compensatory increase in the nonenzymatic AOS capacity that followed from increased levels of urea and uric acid: by 93.4% ($p < 0.001$) and 97.3% ($p < 0.001$) in the brain and by 36% ($p < 0.001$) and 98% ($p < 0.001$) in the blood plasma; the urea concentration in the liver increased by 17.4% ($p < 0.001$), and the uric acid concentration did not change.

Low temperatures inducing LPO activation caused an increase in the cholesterol concentration in the brain, liver, and erythrocyte membranes by 20% ($p < 0.05$), 51.6% ($p < 0.01$), and 35.8% ($p < 0.05$), respectively, that in turn induced structural rearrangements of membranes.

Accordingly, the main deleterious effects of cold stress are as follows: activation of free radical oxidation due to the displacement of the prooxidant–antioxidant balance towards LPO and inactivation of the intracellular AOS enzymes. Under stress, nonenzymatic AOS is the most efficient defense mechanism against ROS.

The formation of adaptive defense mechanisms is provided by activation of the genetic apparatus and metabolic changes in the cells and by functional changes in virtually all systems (nervous, endocrine, cardiovascular, muscular, respiratory, etc.). It is evident that nonspecific regulators of the physiological and biochemical processes and of gene expression play a crucial role in adaptation to stressful factors [13, 38, 39]. We suggest that DSIP acts as such nonspecific regulator. This hypothesis is supported by the ability of DSIP to inhibit ROS production and to increase AOS efficiency (Tables 1-5).

The anti-stress effect of DSIP at low temperatures consisted in inhibiting xanthine oxidase in the brain and liver, which triggered free radical reactions and induced accumulation of toxic by-products, ROS, by 30% ($p_1 < 0.001$) and 29.4% ($p_1 < 0.001$), respectively, compared to cold-treated animals; the enzyme activity was by 30.7% ($p < 0.001$) and 36.5% ($p < 0.001$) higher in the brain and liver, respectively. The myeloperoxidase activity in blood neutrophils, the most important bactericidal system, after DSIP injection increased by 49.3% ($p_1 < 0.01$), compared to cold stress, and was close to the control value.

We think that the most important protective effect of DSIP is associated with its ability to increase AOS capacity involving different enzymes and nonenzymatic components. Although the DSIP molecule did not inhibit free radical processes [40], the antioxidant effect of DSIP through the activation of major antioxidant enzymes and

compounds was clearly pronounced. We showed that DSIP injection before cold stress primarily activated specific defense reactions in the brain and liver, followed by their induction in the blood. This finding is consistent with the data of Krupennikova et al. [41], who showed that DSIP produced an anti-stress effect under cold stress at its concentrations in the brain exceeding its physiological level by a factor of 6. DSIP normalized the AOS that was manifested by a considerable increase in the activities of SOD and catalase: by 46.8% ($p_1 < 0.01$) and 91.7% ($p_1 < 0.001$) in the brain, by 132% ($p_1 < 0.001$) and 121.6% ($p_1 < 0.001$) in the liver, and by 104% ($p_1 < 0.001$) and 25.3% ($p_1 < 0.05$) in the erythrocytes, respectively, compared to cold stress. These parameters did not differ significantly from the control. Under cold stress, DSIP stimulated glutathione peroxidase and glutathione reductase and increased the concentration of reduced glutathione by 93% ($p_1 < 0.01$), 38% ($p_1 < 0.01$), and 16.8% ($p_1 < 0.05$) in the brain, by 67% ($p_1 < 0.01$), 64% ($p_1 < 0.001$), and 103.2% ($p_1 < 0.001$) in the liver and by 30% ($p_1 < 0.001$), 56.5% ($p_1 < 0.01$), and 30% ($p_1 < 0.001$) in the erythrocytes, compared to cold stress alone. Virtually all parameters reached control values; the glutathione reductase activity in the liver became 23.2% higher than in the control ($p < 0.01$); this may be regarded as an adaptive reaction directed at maintaining the optimum level of reduced glutathione rapidly utilized under stress. The concentration of reduced glutathione in the brain also exceeded (by 26%; $p < 0.01$) its concentration in the control. A potent stimulating effect of DSIP on the activity of the glutathione peroxidase system under stress suggests the prevalence of the glutathione mechanism of DSIP action that is of crucial importance for the organism.

We have shown that DSIP acts both on the extracellular enzyme systems and on the nonenzymatic mechanisms involved in AO defense under stressful conditions. Under cold stress, DSIP caused a decrease in the ceruloplasmin oxidase activity in blood plasma by 28.7% ($p_1 < 0.001$), as compared to cold stress; however, this parameter was higher than in the control group by 19.4% ($p < 0.05$). DSIP injected before cold treatment led to the restoration of the original level of MSM in the blood plasma: both a peptide and a non-peptide components decreased by 50% ($p_1 < 0.001$) and 31.6% ($p_1 < 0.001$), respectively, as compared to cold stress. The distribution coefficient k increased by 37.6% ($p_1 < 0.001$) and reached normal values. However, DSIP prevented a decrease in MSM AO in tissues, probably due to stabilization of cellular membranes. Upon DSIP injection to cold-treated rats, the level of non-peptide and peptide MSM fractions in the liver increased by 30.2% ($p_1 < 0.001$) and 17% ($p_1 < 0.05$), respectively, in comparison with the same parameters in the cold-treated rats, and did not differ significantly from the control. The coefficient k also did not change significantly. In the brain of animals exposed to cold, DSIP injection increased the non-peptide and peptide MSM components by 29% ($p_1 < 0.001$) and 21.8% ($p_1 < 0.001$), respectively, as compared to the same parameters in rats under stress. The level of MSM non-peptide fraction was by 9% higher ($p < 0.001$) and of the peptide fraction, by 11% lower ($p < 0.05$) than in the control. The coefficient k indicating changes in the MSM fraction decreased by 17.8% ($p < 0.001$) in the brain, as compared to the control, and did not differ from the corresponding value under stress.

The ability of DSIP for regulating the prooxidant–antioxidant balance in tissues and blood of normal and cold-exposed rats by changing the amount of the low-molecular-weight compounds, which show both anti- and prooxidant properties in a concentration-dependent manner, deserves special attention. The injection of DSIP to cold-treated animals decreased the levels of urea and uric acid by 43% ($p_1 < 0.001$) and 26.4% ($p_1 < 0.001$), respectively, in the brain and by 24% ($p_1 < 0.01$) and 33.2% ($p_1 < 0.001$) in the blood plasma; in the liver, the urea concentration decreased by 15.4% ($p_1 < 0.01$), as compared to cold stress; the concentration of uric acid remained constant. Thus, DSIP restored the normal level of urea in tissues and blood plasma, while the uric acid concentration in the brain and blood plasma was by 45.2% ($p < 0.001$) and 32.2% ($p < 0.01$) higher, respectively, than in the control and did not differ significantly from the control in the liver. The mode of action of DSIP on nitrogen metabolism in tissues and blood is probably realized through the modification of the structure, activity, and optimum conditions for the enzymes involved in the synthesis and degradation of urea and uric acid as well as by the regulation of their filtration, reabsorption, and secretion from the organism.

In all probability, DSIP is involved in the regulation of lipid metabolism, thus affecting membrane structure. The injection of DSIP before cold treatment prevented excess accumulation of cholesterol and maintained it at the control level in the brain, liver, and erythrocyte membranes, decreasing its concentration by 27.5% ($p_1 < 0.01$), 37% ($p_1 < 0.05$), and 39% ($p_1 < 0.01$), respectively, compared to cold stress.

In summary, injection of DSIP before cold treatment restores the prooxidant–antioxidant balance disturbed by cold stress by increasing the capacity of the endogenous AO defense mechanisms in the tissues and blood, the glutathione peroxidase system in particular, and of nonenzymatic reactions.

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