Effect of δ-Sleep-Inducing Peptide on Rat Erythrocyte Membrane Structure in Cold Stress

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δ-Sleep-inducing peptide (1 μg/ml) added to erythrocyte suspension from intact rats enhanced quenching of membrane tryptophanyl fluorescence with pyrene and increased the microviscosity of zones of protein-lipid contacts. Microviscosity and polarity of membrane lipid phase remained unchanged. Exogenous δ-sleep-inducing peptide increased the negative surface charge of the erythrocyte membrane. During cold stress, the efficiency of tryptophane fluorescence quenching with pyrene decreased and microviscosity of protein-lipid contacts decreased, while microviscosity of lipid layer of the erythrocyte membranes did not change; polarity of deep membrane layers and negative surface charge increased. δ-Sleep-inducing peptide normalized the efficiency tryptophane fluorescence quenching with pyrene and membrane microviscosity, polarity, and surface charge.

Key Words: *δ-sleep-inducing peptide; stress; erythrocytes; membranes*

Molecular mechanisms of the effects of regulatory peptides on cell membranes are now extensively studied. Neuropeptides regulate ionic membrane permeability via interaction with ionic channel molecule or formation of ionic channel due to incorporation of the neuropeptide molecule into lipid matrix; moreover, they form complexes with phospholipids, which modify membrane fluidity [11]. δ-Sleep-inducing peptide (DSIP) attracts special attention as an antistress and hypnogenic brain modulator, adaptogen with a polyfunctional effect on the organism under normal and extreme conditions, including the membrane-stabilizing effect [3], and *in vitro* modifying the structure of erythrocyte membranes (EM) in rats.

Regulation of various biochemical processes in the body is mediated through cell membranes, specifically, through modulation of their physicochemical status. Changes in the lipid phase of cell membrane, *e. g.*, changes in lipid composition, oxidation of membrane lipids, microviscosity, polarity, *etc.*, play an important role in the above-mentioned processes [13]. Free-radical processes and biomembrane damage play a key role in nonspecific reaction of the organism to extreme environmental factors [2]. We investigated the effect of DSIP on the structure and surface charge of rat EM under normal conditions and during cold stress.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats weighing 150-180 g in winter. Intact animals were kept in a vivarium at 18-20°C on a standard ration (control); stressed animals were kept for 3 days in a cold chamber at 0-4°C (cold stress). The period of 1-3 days in a cold chamber was defined as a stress by the stress and adaptation tests [4,5,10,12]. Experimental and control animals were decapitated in the morning 3 days after the beginning of experiment, blood was collected in heparinized tubes. After removal of the plasma, erythrocyte precipitate was resuspended in 10 ml 0.15 M NaCl containing 0.01 M Tris-HCl (pH 7.4),

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centrifuged for 15 min at 3000 rpm, washed 3-fold, and suspensions in buffered (pH 7.4) normal saline with equal protein content (0.5 mg/ml sample) were prepared from the resultant compact erythrocyte precipitate. Protein content was measured by the method of Lowry [14].

The effect of DSIP on the structure of rat EM was studied in *in vitro* experiments. Erythrocyte suspension was preincubated with 1 μg/ml DSIP for 30 min at 26°C (DSIP was synthesized at M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry) and with fluorescent probes pyrene and 1-anilinonaphthalene-8-sulfonate (ANS). In control samples pyrene or ANS fluorescence in membrane was examined without adding any other substances.

The structure of hydrophobic area of EM was evaluated by the intensity of membrane tryptophanyl fluorescence, pyrene eximerization, and the efficiency of tryptophane fluorescence quenching with pyrene measured at 25°C on a Hitachi 650-60 spectrofluorimeter in 0.1-cm cuvettes positioned at a 45° angle. Tryptophane fluorescence of EM was measured at excitation λ=282 nm and emission λ=330 nm. Then pyrene was added to EM suspension (final concentration 7 μM) and after 1 min (time of complete dissolving of pyrene in membrane lipid phase) tryptophane fluorescence was measured again, and the intensities of monomer (F_m) and eximer (F_e) pyrene fluorescence were measured at emission maximums of 393 and 470 nm, respectively. The height of fluorescence peak (F) in this case was lower than at first measurement (F_0) due to tryptophane fluorescence quenching with pyrene. Submerging of membrane proteins in the lipid matrix was evaluated by the efficiency of energy transfer from tryptophanyls to pyrene equal to $F_0/(F_0-F)$. Pyrene eximerization coefficient $K_e=F_e/F_m$ was measured at excitation wavelengths of 334 and 282 nm. Membrane microviscosity is reciprocal to the pyrene eximerization coefficient. The polarity of lipid environment of the probe was evaluated by the ratio of fluorescence intensity of two monomeric forms F_{372}/F_{393} in fine pyrene spectrum structure at excitation λ=334 nm [7].

The status of membrane surface was studied with ANS fluorescent probe [8] in concentrations of 10, 20, and 30 μM. Fluorescence intensity was studied at excitation λ=370 nm and emission λ=480 nm. The intensity of membrane-bound ANS fluorescence and the surface charge are reciprocal values [9].

Significance of difference between experimental and control groups was evaluated by Student's t test.

RESULTS

Physical characteristics of rat EM recorded by pyrene fluorescent probe were reliably changed by DSIP (Table 1). The $F_0/(F_0-F)$ ratio decreased by 17.1%, which indicates a higher efficiency of membrane tryptophanyl fluorescence quenching by pyrene in EM from intact animals under the effect of DSIP. The degree of protein submerging in the lipid phase can be evaluated by the efficiency of tryptophane fluorescence quenching with pyrene [8]. From such a viewpoint, we can speak about translocation of proteins into hydrophobic bilayer. The degree of pyrene eximerization is inversely proportional to energy transfer efficiency [8]. In our experiments DSIP reduced pyrene K_e (282) in intact rat EM by 20.2%, which indicated an increase in the microviscosity of the protein-lipid interface (Table 1). Under these conditions the microviscosity of lipid bilayer evaluated by K_e (334) for pyrene and polarity of its microenvironment in EM remained virtually unchanged (Table 1). The decreased of ANS fluorescence intensity by 9.1, 12.3, and 13.3% for probe concentrations of 10, 20, and 30 μM, respectively, under the effect of DSIP is an indirect evidence of the immediate reaction between DSIP and EM (Table 1). Being an amphyphilic molecule, DSIP interacts with lipid membrane increasing its negative surface charge,

TABLE 1. Effect of DSIP on the Structure and Surface Charge of EM in Intact Rats and Rats Exposed to Cold (*M±m*, *n*=16-32)

Parameter	Control	DSIP	Cold stress	Cold stress+DSIP
$F_0/(F_0-F)$	3.83±0.11	3.18±0.09*	4.91±0.26*	3.79±0.12+
$K_y=F_y/F_l$ (282)	1.57±0.05	1.25±0.05*	1.97±0.04*	1.53±0.03+
$K_y=F_y/F_l$ (334)	0.67±0.02	0.69±0.01	0.62±0.02	0.62±0.01
F_{372}/F_{393} (334)	1.22±0.04	1.14±0.06	1.44±0.02*	1.33±0.02*+
ANS fluorescence intensity				
ANS, μM				
10	1.65±0.01	1.50±0.01*	1.43±0.02*	1.62±0.02+
20	1.85±0.02	1.62±0.01*	1.57±0.02*	1.75±0.04*+
30	2.06±0.03	1.78±0.01*	1.68±0.03*	1.89±0.04*+

Note. *p<0.05 *vs.* the control, +p<0.05-0.001 *vs.* cold stress.

which reduces binding of ANS because of direct competition between these anions.

Cold stress leads to LPO activation and reduces activity of the erythrocyte antioxidant system, which enhances EM permeability. Exposure to a stress factor impairs the structure of membrane proteins and lipid bilayer. Measurement of the efficiency of nonradiation energy transfer from tryptophane residues to pyrene in erythrocyte membrane from stressed rats revealed a significant (by 28.1%) decrease in the $F_0/(F_0\text{-}F)$ parameter in comparison with the control, which indicates translocation of erythrocyte proteins to the surface of the lipid bilayer and/or accumulation of associated proteins in EM of stressed rats (Table 1). Changes in the protein-lipid interactions in rats exposed to cold stress are in good correlation with the formation of protein aggregates under the effect of free radicals [6,8]. Pyrene K_c (282) in EM of stressed rats increased by 25.1% in comparison with the control (Table 1), which indicates decreased microviscosity of the protein-lipid interface and disturbed protein-lipid interactions in EM. The structural parameter characterizing the relative microviscosity of lipid bilayer in EM changed negligibly in cold stress (Table 1). Measurements of a fine structure of pyrene fluorescence spectrum in the short-wave range showed increased polarity of EM lipid phase (by 18.3%) in experimental group in comparison with the control (Table 1), probably due to formation of polar LPO products in membranes [6]. Apart from changes in the lipid layer, LPO was associated with changes in the surface charge of EM from animals exposed to stress. The intensity of ANS fluorescence at probe concentrations of 10, 20, and 30 μM decreased by 13.5, 15, and 18%, respectively, in comparison with the control, which indicates an increase in the negative membrane surface charge and can be explained by the formation of polar oxidation products, in particular carboxyls, on the surface of EM during LPO (Table 1).

DSIP (1 μg/ml) *in vitro* prevented unfavorable structural changes in EM from rats exposed to cold stress. The effect of DSIP on EM is determined by its influence on the surface charge, polarity, microviscosity of protein-lipid interface, and structure of membrane bilayer modified under conditions of LPO. DSIP decreased the $F_0/(F_0\text{-}F)$ parameter in erythrocyte suspension in comparison with cold stress, indicating a higher efficiency of tryptophane fluorescence quenching with pyrene and hence, a deeper protein localization in the membrane (Table 1). This parameter reached the control level. Dissociation of protein is accompanied by complexes enhanced energy transfer from integral proteins to pyrene; simultaneously, the mean distance between pyrene molecules increased, while their eximerization decreased. DSIP decreased pyrene K_c (282) in EM of experimental rats by 22.4% in comparison with cold stress, and it did not differ from the control (Table 1). Addition of DSIP to erythrocyte suspension from experimental rat normalized membrane structure: it decreased polarity of pyrene microenvironment by 8.2% in comparison with cold stress; this parameter was 8.7% higher than in intact animals; microviscosity of lipid bilayer did not change. DSIP enhanced ANS fluorescence in EM of rats exposed to cold stress at probe concentrations of 10, 20, and 30 μM by 13.8, 11.4, and 12.4%, respectively, in comparison with stress, which implies a decrease in the membrane negative surface charge virtually to the control level. ANS fluorescence intensity in samples with probe concentrations of 20 and 30 μM remained below the control by 5.3 and 7.9%, respectively (Table 1).

Thus, our experiments demonstrated membrane-stabilizing effect of DSIP in intact rats and in animals exposed to cold stress: it normalized the structure of membrane proteins and the state of protein-lipid interface, prevented accumulation of polar compounds in the deep layers, and modification of surface layer in EM.

REFERENCES

1. I. P. Ashmarin and M. F. Obukhova, *Vestn. Ross. Akad. Med. Nauk,* No. 10, 28-34 (1994).
2. V. A. Baraboi, *Uspekhi Sovrem. Biol.,* **111,** No. 6, 923-931 (1991).
3. T. I. Bondarenko, *Homocarnosine Metabolism in the Brain of Animals of Different Age and under Extreme Environmental Conditions*, Author's Abstract of Doct. Biol. Sci. Dissertation, Moscow (1990).
4. T. I. Bondarenko, A. A. Krichevskaya, E. Yu. Krupennikova, and I. I. Mikhaleva, *Fiziol. Zh. SSSR,* **71,** No. 3, 279-282 (1985).
5. T. I. Bondarenko, A. A. Krichevskaya, I. V. Sheikina, and E. V. Kiryukhina, *Ukr. Biokhim. Zh.,* **62,** No. 5, 34-38 (1990).
6. Yu. A. Vladimirov, *Biofizika,* **32,** No. 5, 830-843 (1987).
7. Yu. A. Vladimirov and G. E. Dobretsov, *Fluorescent Probes in Studies of Biological Membranes* [in Russian], Moscow (1980).
8. G. E. Dobretsov, *Fluorescent Probes in Studies of Cells, Membranes, and Lipoproteins* [in Russian], Moscow (1989).
9. G. N. Zatsepina, T. V. Vengrus, and N. N. Goryunov, *Biofizika,* **38,** No. 6, 1098-1103 (1993).
10. E. Yu. Krupennikova, R. Yu. Yukhananov, I. I. Mikhaleva, and T. I. Bondarenko, *Neirokhimiya,* **2,** No. 5, 190-193 (1986).
11. S. A. Muranevich, *Ros. Fiziol. Zh.,* **79,** No. 4, 9-29 (1993).
12. T. I. Bondarenko and I. I. Mikhaleva, *Delta-Sleep Inducing Peptide. Theoretical and Applied Aspects,* Rostov-on-Don (1992), pp. 6-7.
13. E. B. Burlakova, G. V. Arkhipova, M. I. Djalyabova, *et al., Biophysical and Biochemical Information Transfer in Recognition,* New York (1979), pp. 583-594.
14. O. H. Lowry, N. I. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.,* **193,** No. 1, 265-275 (1951).