DOI 10.1007/s10517-016-3186-8

Effect of Delta Sleep-Inducing Peptide on Functional State of Hepatocytes in Rats during Restraint Stress

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 160, No. 10, pp. 416-419, October, 2015
Original article submitted March 6, 2015

We studied the effect of delta sleep-inducing peptide (40, 120, and 360 μg/kg intraperitoneally, 1 h before the experiment) on free radical oxidation in the liver, aminotransferase activity, and total serum protein content in male Wistar rats during restraint stress. Treatment with the peptide in a dose of 40 μg/kg increased catalase and superoxide dismutase (SOD) activities and malonic dialdehyde (MDA) concentration in the liver homogenate of animals subjected to acute stress. No significant changes were found after administration of this peptide in other doses. Under conditions of chronic stress, the peptide in a dose of 40 μg/kg caused the most pronounced effect. Catalase and SOD activities and MDA concentration decreased, while aminotransferase activity and protein content remained unchanged under these conditions. Administration of the peptide in a dose of 120 μg/kg was accompanied by a decrease in SOD activity and MDA concentration, increase in total protein content, and reduction of AST activity. Increasing the peptide dose to 360 μg/kg abolished its effects.

Key Words: *delta sleep-inducing peptide; restraint stress; liver; free radical oxidation; serum aminotransferases*

The mechanisms of adverse effects of stress on organs and tissues of the body (*e.g.*, functional state of the liver) are extensively studied. Stress is accompanied by changes in activities of cytochrome P450 family enzymes [9], development of steatosis and nonalcoholic fatty liver disease [10], and induction of hepatocyte apoptosis due to enhanced expression of Fas receptors and migration of NK cells into the liver [7]. Intensified generation of ROS inducing LPO in cell membranes is a mechanism of stress-induced injury to hepatocytes [13]. Activation of this process contributes to an increase in TNF production by Kupffer's cells, which triggers the superoxide cytotoxic mechanism [3]. Therefore, the liver is highly sensitive to stress exposures [6,14].

The negative psychological evaluation of any unavoidable factor plays an important role in the emotional stress response to pain stimulation or immobilization [5]. Hence, analysis of the role of regulatory peptides in the stress reaction and associated changes in the body attracts much attention. Published data show that delta sleep-inducing peptide (DSIP) possesses the antioxidant and anxiolytic properties and increases the survival rate of stressed animals [1,2].

This work was designed to study the effect of DSIP on functional state of hepatocytes in rats during acute and chronic restraint stress (RS).

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=100) weighing 250-280 g. The animals were divided into groups (10 specimens per group). They were maintained under standard vivarium conditions and had free access to water and food (12:12-h light/dark regimen).

DSIP, (NH2)Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu(COOH), was synthesized at the Research Institute of Chemistry (St. Petersburg State University). The peptide was dissolved in physiological saline and injected intraperitoneally (40, 120, and 360 μg/kg)

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60 min before each stress exposure. Control animals received an equivalent volume of physiological saline (1 ml/kg).

RS was produced by fixation of the animal in narrow individual plastic tubes. The duration of immobilization during acute stress was 4 h. Under conditions of chronic stress, the animals were subjected to 2-h daily immobilization for 5 days. By the end of the experiment, all animals were killed by bleeding (blood withdrawal from the right ventricle of the heart) under ether anesthesia.

Activities of ALT and AST, total protein content, and glucose concentration in blood serum were measured on a Vitalit 1000 biochemical analyzer using Vital regents.

The liver was homogenized in ice-cold physiological saline and centrifuged. The content of LPO products and activities of antioxidant enzymes were measured in the supernatant. LPO intensity was evaluated by malonic dialdehyde (MDA) concentration that was measured on an Apel 330 PD spectrophotometer using TBK-Agat reagents. Activities of superoxide dismutase (SOD) and catalase were measured spectrophotometrically. The total antioxidant activity (TAA) was evaluated by the method based on inhibition of ascorbate-induced and iron-induced oxidation of Tween-80 to MDA. The assay was conducted on a BTS-330 biochemical analyzer.

Between-group differences were evaluated by Mann–Whitney test and Student's *t* test (depending on the distribution of data in the sample).

RESULTS

Acute RS produced a potent effect on the analyzed indexes (Table 1). Control animals were characterized by an increase in activities of AST (by 120%, $p<0.001$) and ALT (by 58%, $p<0.01$) in blood serum and significant decrease in the total protein content (by 5%, $p<0.01$ compared to intact specimens). Glucose concentration increased by 28% ($p<0.05$), which is typical of acute stress response and confirmed sufficient strength of the stress factor. We revealed a RS-induced increase in activities of catalase (by 32%, $p<0.05$) and SOD (by 39%, $p<0.05$) and MDA content (by 43%, $p<0.05$) in the liver homogenate. These indexes increased more significantly after injection of DSIP in a dose of 40 μg/kg. Catalase and SOD activities increased by 20 and 36%, respectively ($p<0.05$). The increase in MDA concentration (by 23%, $p<0.05$) was accompanied by a decrease in TAA (by 13%, $p<0.05$). Administration of the peptide in a dose of 360 μg/kg was followed by a significant decrease in AST activity (by 38%, $p<0.01$) and SOD activation (by 22%, $p<0.05$ compared to the control). These effects were not observed after treatment with DSIP in a dose of 120 μg/kg.

Similar to acute stress, chronic RS caused a pronounced change in study indexes. Stressed animals of the control group were characterized by an increase in AST activity (by 49%, $p<0.01$) and significant decrease in the total protein content (by 4%, $p<0.05$ compared to intact specimens). No significant changes were found in ALT activity. We revealed an increase in activities of catalase (by 29%, $p<0.05$) and SOD (by 63%, $p<0.01$) and amount of MDA (by 48%, $p<0.05$) in the liver homogenate. DSIP in a dose of 40 μg/kg produced the most pronounced effect, which was manifested in a decrease in activities of catalase (by 48%, $p<0.001$) and SOD (by 38%, $p<0.001$; level of intact specimens). The decrease in MDA level (by 44%, $p<0.001$) was accompanied by the increase in TAA (by 26%, $p<0.05$). The remaining indexes remained unchanged in animals of this group. Increasing the dose of this peptide to 120 μg/kg was accompanied by a decrease in SOD activity (by 37%, $p<0.001$) and MDA level (by 44%, $p<0.05$) in the liver homogenate. An increase in the total protein content (by 5%, $p<0.05$) was accompanied by a decrease in AST activity (by 23%, $p<0.05$) in blood serum. These effects were abolished with a further increase in the dose of DSIP to 360 μg/kg.

Our results indicate that DSIP in a dose of 40 μg/kg has a strong effect on free radical oxidation in the liver. The direction of DSIP-induced changes depends on the duration of stress exposure. We revealed an increase in activity of antioxidant enzymes and concentration of MDA under conditions of acute RS. These surprising results require further investigations. By contrast, during chronic RS these indexes were shown to decrease to the level observed in intact animals, which is consistent with published data on the anti-stress effect of DSIP [1]. The stress-protective effect of DSIP can be associated with its ability to decrease the basal level of ACTH, which serves as a major component of the stress-realizing system [11]. Activation of the hypothalamic–pituitary–adrenal system has a strong effect on LPO processes in the liver [15].

The observed discrepancies are probably due to polyfunctional biological properties of regulatory peptides and dose-dependent effects of these compounds (as reported for DSIP) [2].

Administration of DSIP in a dose of 120 μg/kg under conditions of chronic stress was followed by an increase in the total protein content. This effect was probably mediated by the following mechanism. Published data show that DSIP activates ribonucleotide reductase in the spleen of mice. This enzyme plays a key role in the synthesis of DNA and protein [12]. A similar activation probably occurs in hepatocytes,

TABLE 1. Effects of DSIP during Acute and Chronic RS (*M*±*m*)

Parameter	Intact animals	Stress exposure			
		control	DSIP, μg/kg		
			40	120	360
Acute RS					
Blood serum					
ALT, U/liter	53.3±3.7	84.4±10.4^{+}	96.2±6.0	73.3±3.9	65.8±7.6
AST, U/liter	123.6±4.9	271.9±32.8^{+}	321.6±28.2	306.3±35.0	169.6±14.9*
Total protein, g/liter	73.3±0.7	69.3±1.2^{+}	71.6±1.9	67.8±1.4	69.1±0.8
Glucose	10.3±0.4	13.1±0.7^{+}	14.0±1.7	10.1±1.4*	12.9±1.1
Liver homogenate					
MDA, μmol/ml	4.1±0.7	5.9±0.5^{+}	7.3±0.4*	6.7±0.2	6.4±0.5
Catalase, μcat/liter	4.4±0.4	5.7±0.5^{+}	6.9±0.2*	6.1±0.7	6.1±0.3
SOD, arb. units	3.4±0.6	4.7±0.3^{+}	6.4±0.8*	5.0±0.4	5.7±0.3*
TAA, %	33.3±1.8	30.0±1.4	26.0±0.5*	26.7±1.2	27.2±1.2
Chronic RS					
Blood serum					
ALT, U/liter	38.0±3.5	40.1±8.0	41.6±3.8	39.0±6.8	44.5±7.8
AST, U/liter	121.1±5.0	180.0±18.0^{+}	196.2±15.0	138.4±10.2*	151.6±11.2
Total protein, g/liter	73.7±0.6	70.7±1.1^{+}	70.2±1.4	73.9±0.8*	72.2±1.2
Glucose					
Liver homogenate	4.5±0.7	6.7±0.5^{+}	3.8±0.4*	4.9±0.6*	6.8±0.4
MDA, μmol/ml	4.6±0.4	5.9±0.4^{+}	3.1±0.2*	5.1±0.4	5.6±0.4
Catalase, μcat/liter	3.7±0.6	6.0±0.3^{+}	3.7±0.3*	3.8±0.3*	5.2±0.7
SOD, arb. units	32.3±1.9	27.1±1.7^{+}	34.2±1.3*	29.8±1.3	26.1±0.7

Note. p<0.05-0.001 in comparison with *the control group, $^{+}$intact animals.

which promotes an increase of the total protein content in blood serum.

The question arises: whether DSIP can interact with CNS structures after its intraperitoneal injection? DSIP crosses the blood–brain barrier by passive diffusion or due to a special mechanism of transport into the bottom of the fourth ventricle [8]. The influence of this peptide on peripheral organs and tissues is poorly understood. There are no data on the existence of specific receptors for DSIP [1]. However, DSIP can produce a direct effect on cell membranes (*e.g.*, erythrocyte membranes) [4].

We conclude that a hepatoprotective effect of DSIP in rats during acute and chronic RS is associated with the inhibition of cytolytic processes, normalization of protein synthesis in hepatocytes, and modulation of free radical oxidation.

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