Effect of Delta Sleep-Inducing Peptide on Macromolecule Biosynthesis in Brain Tissue of Stressed Rodents

M. G. Makletsova, I. I. Mikhaleva*, I. A. Prudchenko*, and G. T. Rikhireva**

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For evaluation of the nature of adaptogenic properties of delta sleep-inducing peptide we studied the effect of this substance on macromolecule biosynthesis in the brain of rats and mice exposed to burn injury and psychoemotional stress, respectively. Anabolic activity of delta sleep-inducing peptide depended on the purpose of adaptation corresponding to the type of stress.

Key Words: *delta sleep-inducing peptide; stress; macromolecule biosynthesis*

Delta sleep-inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu, DSIP) is an endogenous neuromodulator with a wide range of biological properties [4-6]. This peptide increases organism's resistance to stress, alleviates stress-induced abnormalities, and maintains metabolic processes at the physiological level. Deltaran created on the basis of DSIP was patented in Russia. However, molecular mechanisms of biological activity of DSIP, including antistress and adaptogenic properties, are poorly understood.

Activation of macromolecule biosynthesis plays an important role in adaptation to stress. This process occurs after a short catabolic stage of stress and provides adaptive structural-and-functional changes in biochemical systems. To understand adaptogenic properties of DSIP, it is important to evaluate the effect of this peptide on biosynthetic processes. Previous studies showed that DSIP modulates biosynthesis of proteins and nucleic acids. Addition of this peptide to the culture medium increases DNA concentration in growing cells by 30-40% [5]. The study of ultrastructural characteristics of rat brain cortex showed that DSIP activates nuclear apparatus, rough and smooth endoplasmic reticulum [1]. Here we studied the effect of intraperitoneally injected DSIP on macromolecule biosynthesis in the brain of animals exposed to 2 types of stress (thermal burn in rats, psychoemotional stress in mice).

MATERIALS AND METHODS

DSIP was obtained by the method of Fmoc solid phase peptide synthesis on a Biosearch 9500 automatic synthesizer. The end product was purified by gel filtration on G-15 Sephadex. High-performance liquid chromatography was performed with a reversed phase sorbent [3]. Burn injury was modeled on outbred male albino rats weighing 200-220 g. Thermal burn of the skin was induced as described elsewhere [2]. The animals received intraperitoneal injection of DSIP in a dose of 120 μg/kg 1 h after the incidence of thermal trauma.

The influence of DSIP on biosynthetic processes was estimated by changes in brain polyamine

Laboratory of Clinical Neurochemistry, Institute of Neurology, Russian Academy of Medical Sciences; *M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences; **N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow

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concentration and incorporation of label from ornithine into spermine and spermidine. L,D-[1,4-^{14}C]-ornithine (specific radioactivity 661 μCi/mg) served as a labeled precursor. The label was administered intracisternally 1 h before decapitation. Spermine and spermidine served as the standards (Sigma). Incorporation of label from ornithine into polyamines was assayed by the method of homogeneous counting with dioxane scintillator on a SL 300 liquid scintillation counter and expressed in nmol/min per nmol spermine and spermidine. The results were analyzed by Student's *t* test.

Experiments with psychoemotional stress were performed on 90 male SHK mice weighing 18-22 g. Psychoemotional stress was produced by 30-min playback of alarm call recordings accompanied by flashlight and intermittent grating noise. The mice were divided into 4 groups (1 control group and 3 experimental groups). Control animals received injection of physiological saline. The animals of experimental group 1 (DSIP) received DSIP. The animals of experimental group 2 (Stress) were exposed to stress 1 h after injection of physiological saline. The animals of experimental group 3 (DSIP+ stress) received injection of DSIP 1 h before stress. The rate of total protein synthesis in brain tissue of mice was measured by the radioisotope method. L-[u-^{14}C]-Lysin (specific activity 287 Ci/mol) served as a labeled precursor. The label was injected intraperitoneally 1 h before decapitation. Radioactivity of samples was measured on a Delta 300 scintillation counter (Tim Analitic). The absolute value of L-[u-^{14}C]-lysin incorporation into brain cell proteins of control mice was 15,780 ppm per g wet tissue.

RESULTS

Thermal burn was accompanied by eschar formation. Sloughing in control animals was observed on days 15.0±0.9. Single treatment with DSIP after burn injury shortened the time-to-sloughing (9.0±0.3 days). The degree of burn wound healing was estimated by measuring the area of wound surface (Table 1). DSIP administration significantly decreased the wound surface area on days 14, 21, and 30 (by 29, 32, and 53%, respectively, compared to the control).

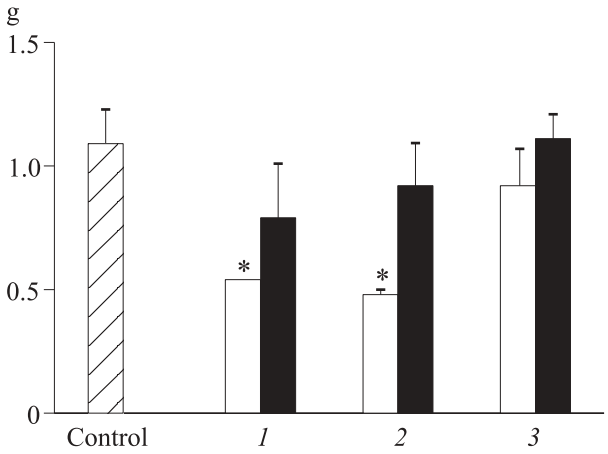

Fig. 1. Effect of delta sleep-inducing peptide (DSIP) on the weight of the spleen during burn stress: after 2 h (*1*), 1 day (*2*), and 3 days (*3*). Light bars, animals of the burn-stress group; dark bars, DSIP administration after burn stress; shaded bars, intact animals (control).

The measurement of the weight of the spleen is an approach to study organism's adaptive capacity. The weight of the spleen sharply decreased 2 h after the incidence of burn injury. The weight of the spleen returned to normal in burned animals of the DSIP group (Fig. 1).

Spermine and spermidine are involved in the regulation of protein biosynthesis, reproduction of genetic information, and growth. Spermidine concentration in the cerebral hemispheres remained unchanged 2 h and 1 day after the incidence of burn injury (Table 2). DSIP administration to burned animals had no effect on spermidine concentration during these periods. Spermidine concentration decreased by 50% on day 3 after burn injury, which reflects profound changes in protein biosynthesis in the cerebral cortex. Spermidine concentration 6-fold increased in DSIP-receiving animals on day 3 after burn injury. These data show that DSIP produces an adaptogenic effect and increases protein synthesis in the brain and whole organism.

Spermine concentration in the cerebral cortex remained unchanged over the 1st day after burn

TABLE 1. Effect of DSIP on the Area of Wound Surface during Burn Injury (mm^2, *M*±*m*, *n*=15)

Series	Time, days					
	1	3	7	14	21	30
Burn	387±35	378±42	395±14	302±33	208±6	157±19
Burn+DSIP	381±40	379±40	375±23	213±10*	135±23*	41±13*

Note. *p<0.05 compared to the control (burn).

TABLE 2. Effect of DSIP on the Concentrations of Spermine and Spermidine in the Cerebral Cortex of Burned Rats (nmol/g tissue, *M*±*m*, *n*=9)

Parameter, group		Time		
		after 2 h	after 1 day	after 3 days
Spermidine	burn	407±29	386±71	211±43*
	burn+DSIP	394±121	347±20	2739±128*
Spermine	burn	152±6	155±34	102±36
	burn+DSIP	169±23	134±23	235±41*

Note. The concentrations of spermine and spermidine in the brain of intact rats are 425±89 and 144±3 nmol/g, respectively. *$p<0.01$ compared to intact animals.

injury, but tended to decrease by the 3rd day (Table 2). DSIP administration was followed by a 60% increase in spermine concentration on day 3 after burn injury, which reflects the anabolic effect of this peptide.

A special series was performed to study the influence of burn injury and subsequent administration of DSIP on incorporation of radioactive label from ornithine into spermine and spermidine during the delayed period after burn injury (day 7, Table 3). Incorporation of radioactive carbon from ornithine into spermine significantly decreased in this period after burn injury, which reflects profound changes in biosynthetic processes (*e.g.*, DNA synthesis). DSIP administration increased incorporation of the label from ornithine into spermine by more than 2 times. Spermidine synthesis underwent similar, but less pronounced changes.

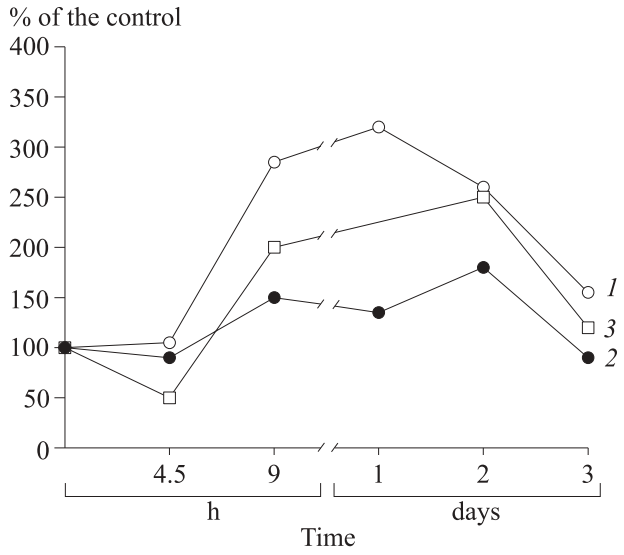

Fig. 2. Rate of total protein biosynthesis in the brain of SHK mice after stress: psychoemotional stress (*1*); DSIP (*2*); and DSIP+psychoemotional stress (*3*). The rate of biosynthesis in control animals is taken as 100%. Zero point in the abscissa, DSIP injection.

The increase in polyamine biosynthesis in DSIP-treated animals exposed to burn stress can be considered as a mechanism of adaptive activity of this substance.

The rate of total protein biosynthesis in brain tissue of SHK mice 9 h after psychoemotional stress increased 3-fold, but returned to normal by the 3rd day after treatment. The rate of protein biosynthesis in intact animals of the DSIP group increased less significantly than in stressed specimens. Administration of the peptide 1 h before psychoemotional stress alleviated stress-induced activation of protein biosynthesis (Fig. 2). The rate of protein biosynthesis in these animals on day 3 after treatment did not differ from normal. DSIP has a modulatory effect on anabolic processes during acute psychoemotional stress. The protective effect of DSIP is probably associated with deceleration of the synthesis of protein factors involved in the stress response (*e.g.*, prohormones).

Our results suggest that DSIP is involved in the regulation of biosynthetic processes in the brain tissue. DSIP increases the concentrations of spermine and spermidine and normalizes the weight of the spleen in animals exposed to burn stress. This peptide produces an anabolic effect, prevents dystrophy, and activates proliferative processes. DSIP serves as a modulator of biosynthetic systems during psychoemotional stress. The anabolic effect of

TABLE 3. Effect of DSIP on Incorporation of Carbon Isotope from L,D-[1,4-^{14}C]-Ornithine into Polyamines in Brain Tissue of Rats on Day 7 after Burn Stress (ppm/nmol/min, *M*±*m*, *n*=9)

Polyamine	Control (intact rats)	Burn	Burn+DSIP
Spermidine	71±23	49±16	96±24
Spermine	241±52	52±3*	643±115*

Note. *$p<0.01$ compared to the control.

this peptide under various stress conditions probably depends on the purpose of adaptation corresponding to the type of stress.

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