ANALYSIS OF THE MECHANISM OF THE STRESS-PROTECTIVE ACTION OF DELTA SLEEP INDUCING PEPTIDE

R. Yu. Yukhananov, V. V. Rozhanets,
I. I. Mikhaleva, and A. I. Maiskii

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A peptide inducing the δ-phase of sleep (DSIP) has been isolated from rabbit brain during hypnogenic stimulation of the thalamus [11], and its presence in the body has been repeatedly confirmed by radioimmuological and immunohistochemical methods of analysis [3, 6]. The hypnogenic effect of the synthetic peptide when injected parenterally, depending on the actual conditions used, has been both confirmed and denied by several investigators [6]. It has also been demonstrated that this peptide affects circadian rhythms of motor activity and changes neurotransmitter metabolism and enzyme activity [6].

The physiological role of the peptide, it is nowadays considered, may be connected with the mechanisms of adaptation to stess. DSIP normalizes the state of biological membranes during adaptation to cold stress [1], it weakens the intensity of emotional stress evoked by stimulation of the ventromedial hypothalamus [12], and diminishes several manifestations of immobilization stress [2]. The concrete mechanism through which the peptide exerts its action has not been established, but there is reason to suppose that at least some of the effects of the peptide are realized through the participation of the opioid system. The action of the peptide on the structure of sleep and the analgesic action of the peptide are blocked by naloxone [9, 12].

To study the effect of DSIP on stress we measured the concentration of ACTH, δ-endorphin corticosterone, and insulin in the blood plasma and also of Leu- and Met-enkephalins in the brain.

EXPERIMENTAL METHOD

Male laboratory albino rats weighing 180-220 g, kept in the animal house with natural schedule of daylight and darkness and with free access to food and water, were used. Experiments were carried out from November to January. The animals were immobilized for 6 h by Selye's method, fixation being in the prone position. After decapitation the brain was removed and quickly frozen in liquid nitrogen. Peptides were extracted with acetic acid [4]. Blood was collected at the same time and plasma obtained by centrifugation in the presence of EDTA. The adrenals were extracted in the same way as brain tissue. The samples were kept until analysis at −70°C. Immunoreactivity, corresponding to Met- and Leu-enkephalins and β-endorphin, was determined by radioimmunoassay, using serum generously provided by A. D. Dmitriev (All-Union Mental Health Center, Academy of Medical Sciences of the USSR), as described previously [4, 5]. Concentrations of ACTH and insulin were estimated by radioimmunoassay using standard kits from "Amersham." The corticosterone concentration was measured with the aid of antiserum generously provided by G. V. Katsiya (Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR), using ^{3}H-corticosterone from "Amersham." Concentrations were calculated by the transformation method. DSIP (synthesizaed in the Institute of Bioorganic Chemistry, Academy of Sciences of the USSR) was injected intraperitoneally 1 h before the beginning of immobilization, in a dose of 0.1 mg/kg. Control animals received injections of the equivalent volume of 0.14 M NaCl.

Laboratory for the Search for and Study of Agents for Prevention and Treatment of Drug Addictions, Research Institute of Pharmacology, Academy of Meical Sciences of the USSR. Laboratory of Peptide Chemistry, N. N. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 46-47, January, 1990. Original article submitted April 3, 1989.

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TABLE 1. Effect of Immobilization Stress on Hormone and Neuropeptide Levels in Rats (M ± m)

Compound	Intact animals	Immobilization	
		physiological saline	DSIP
Blood plasma			
Corticosterone, μg/ml	6,2±0,2 (4)	24,5±2,1* (4)	16,1±1,7** (3)
β-Endorphin, fmoles/ml	140±22 (7)	266±31** (4)	212±17* (5)
ACTH, pg/ml	59±26 (7)	238±35** (4)	194±42* (5)
Insulin, μE/ml	27±8 (4)	26±7 (4)	17±2 (4)
Adrenals			
Leu-enkephalin, fmoles/mg tissue	46±5 (4)	33±7 (4)	34±9 (4)
Met-enkephalin, fmoles/mg tissue	84±12 (4)	170±28* (4)	168±24* (4)

Legend. *p < 0.05 Compared with intact animals, **p < 0.01 compared with group of rats receiving physiological saline. Number of animals in experiment given in parentheses.

TABLE 2. Effect of DSIP on Concentration of Enkephalins (in fmoles/mg tissue) in Brain of Four Rats Exposed in Stress (M ± m)

Brain region	Neuro-peptide	Intact animals	Immobilization	
			physiological saline	DSIP
Cerebral cortex	Leu-enkephalin	42,6±8,3	61,0±14,4	51,7±2,6
	Met-enkephalin	452±61	486±77	420±57
Striatum	Leu-enkephalin	201±37	157,6±33	103±18
	Met-enkephalin	1616±131	920±130**	720±53*
Thalamus	Leu-enkephalin	167±14	163±24	115±31
	Met-Enkephalin	856±96	680±36	724±30
Medulla + pons	Leu-enkephalin	116±23	97,2±13,6	107±16
	Met-enkephalin	940±159	480±38*	468±37*

Legend. *p < 0.05 Compared with intact rats.

EXPERIMENTAL RESULTS

The data in Table 1 show that immobilizing the animals was accompanied by a significant rise in the plasma levels of corticosterone, ACTH, and β-endorphin, the insulin level remaining unchanged.

Preliminary injection of DSIP lowered the corticosterone level when raised by the action of immobilization stress, but had virtually no effect on the concentrations of β-endorphin and ACTH and on the concentration of enkephalins in the adrenals, although it reduced the plasma insulin concentration a little (Table 1). Corticosterone is known to be the most sensitive marker of the intensity of the response to stress, and its concentration is increased during exposure to different kinds of stress [10]. Elevation of the corticosterone level is under ACTH control. In the present experiments, an increase in the ACTH concentration was accompanied by an increase in the corticosterone concentration, but on injection of DSIP, a fall of the corticosterone level, but not of ACTH, was recorded. It could accordingly be postulated that DSIP partially blocks the action of ACTH on corticosterone secretion. However, direct experiments with injection of exogenous ACTH showed that DSIP does not affect the action of ACTH, but it reduces the stimulating action of exogenous ACTH on corticosterone secretion [7]. Considering that the presence of DSIP has been demonstrated immunohistochemically in the intermediate lobe of the pituitary [14] it can be tentatively suggested that the antistress action of the peptide is partially realized at the pituitary level, although the method of its interaction with ACTH and corticosterone is not yet clear.

During stress the Met-enkephalin level in the striatum and medulla fell considerably (Table 2), in agreement with data on the role of the endogenous opiate system in the response of an organism to stress [8]. The Leu-enkephalin level was unchanged in all structures studied. Evidently the μ-encephalic system is primarily involved in the response of the body to stress, for Met-enkephalin possesses definite affinity for μ-opiate receptors. A similar situation was found during the study of enkephalin concentrations in the adrenals, where the concentration only of Met-enkephalin was increased in response to stress (Table 1). Injection of DSIP had no effect on the concentration of either Leu- or Met-enkephalins. We found that DSIP in experiments in vitro does not interact with opiate receptors. In that case it can be tentatively suggested that the endogenous opiate system does not participate in the realization of the stress-protective action of DSIP, although the possibility cannot be ruled out that DSIP may affect the secretion of other opioid peptides.

Thus, the stress-protective action of DSIP is probably realized through the pituitary-adrenal system. The role of the endogenous opioid system, however, was not confirmed by these experiments.

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