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MECHANISM OF THE ANTISTRESS ACTION OF D-ALA^2-LEU^5-ARG^6-ENKEPHALIN

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KEY WORDS: Leu-enkephalin analog; blood hormones; cAMP; stress.

The literature on peptide regulators contains information on activation of the system of endorphins and enkephalins in stress [8, 9], and it suggests that it may be possible in principle to use analogs of endogenous opioids to regulate the severity of stress changes in the body pharmacologically. However, opportunities for testing this hypothesis in practice have been limited by the very short half-life of opioid peptides *in vivo*. Progress toward overcoming this difficulty has been due largely to increasing their resistance to enzymic degradation by substituting glycine in position 2 of the oligopeptide chain for alanine [11]. The writers have shown that a stable analog of Leu-enkephalin has a marked antistress protective effect [3]. However, the mechanisms of this phenomenon have not yet been explained.

The aim of this investigation was to study the effect of the arginine-containing hexapeptide analog of Leu-enkephalin — D-Ala^2-Leu^5-Arg^6-enkephalin (henceforward called enkephalin) on blood plasma levels of ACTH, cortisol, and hormones of the pituitary-thyroid complex, and the cAMP concentration in adrenal and thymus tissues during stress induced by crushing the soft tissues (CST).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-180 g. CST was reproduced by the method in [1]. Half of all the rats were given an intraperitoneal injection of enkephalin (the product was obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Dr. of Chem. Sci. M. I. Titov), and the remaining animals received physiological saline (control). The rats were decapitated in groups of 8-10 at a time, 60 and 300 min after CST and immediately after decompression, under ether anesthesia, blood was taken, the thymus and adrenals were quickly removed with cold scissors on ice, and pieces of the tissues were frozen in liquid nitrogen, weighed, and homogenized in cold absolute alcohol. After centrifugation, the cAMP concentration in the supernatant was determined by the competitive protein binding method, using kits from Amersham Corporation (England). The plasma ACTH and cortisol levels were determined by radioimmunoassay using kits from CEA-Sorin (France), thyroxine (T_4) and tri-iodothyronine (T_3) were determined with kits from Byk Mallinckrodt (West Germany), and pituitary thyrotropic hormone (TTH) by a kit from Corning (USA). A Tracor Analytic Gamma-spectrometer (USA) and Mark III Beta-scintillation counter (USA) were used. The results were subjected to statistical analysis with the aid of Strelkov's tables [7] and by calculation of the Wilcoxon-Mann-Whitney nonparameteric criterion (P_u).

EXPERIMENTAL RESULTS

During preliminary experiments the optimal dose of enkephalin (1.25 nmole/kg body weight), giving an antistress effect [3], was chosen. CST for 60 min caused a marked increase in ACTH and cortisol concentrations in the plasma of the experimental rats and in the cAMP concentration in the adrenal tissue (Table 1). The cAMP level in thymus tissue after 60 min of CST also was raised, evidently due to stress-induced hypercatecholaminemia, for the lymphocyte membranes are richly supplied with adrenoreceptors [13]. Of the thyroid hormones, a sig-

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TABLE 1. Effect of Enkephalin on Plasma Hormone Levels and cAMP Concentration in Rat Adrenal and Thymus Tissues after CST (M ± m)

Experimental conditions	ACTH, pg/ml	Cortisol, ng/ml	T_4, pg/100 ml	T_3, ng/100 ml	TTH, μU/ml	cAMP, picomoles/g	
						adrenals	thymus
Intact rats	173,75±6,47	5,76±0,69	4,39±0,44	82,63±6,24	12,45±2,5	2899,8±304,8	916,2±275,9
Control (CST for 60 min)	594,79±78,3*	21,36±1,82*	3,50±0,48	70,31±5,9	1,24±0,47*	4301,9±648,37*	2036,39±173,54*
CST 60 min + enkephalin	370,0±61,55*	23,28±2,02*	3,33±0,41	72,41±14,7	4,2±1,12	3039,3±998,87	2222,3±200,59*
Control (CST for 300 min)	290,14±32,4*	26,74±2,4*	1,77±0,22*	36,14±7,06*	1,05±0,42*	4657,6±1077,4†	1550,4±206,93†
CST 300 min + enkephalin	320,03±22,8*	17,96±0,08† $P_U<0,05$	2,45±0,5†	66,94±13,3† $P_U<0,05$	10,83±2,24 $P_U<0,01$	2401,0±142,31 $P_U<0,01$	2484,6±208,23* $P_U<0,01$

Legend. *P < 0.01, †P < 0.05 compared with intact animals, P_u) significance of differences compared with control.

nificant decrease after 60 min of CST was observed only in the case of TTH, in agreement with data in the literature [5], and also evidently due to adrenergic inhibition of TTH secretion at the hypothalamic level [12]. Injection of enkephalin into the rats was accompanied by lowering of the ACTH level and by an increase in the TTH concentration after 60 min of CST compared with the control. After 300 min of CST the plasma cortisol concentration and the cAMP concentration in the adrenals continued to rise. The ACTH concentration remained high, with a tendency toward inhibition. The cAMP level in the thymus fell ($P_u < 0.05$). A marked fall in the levels of all thyroid hormones also was noted.

These changes are characteristic of stress, when the high corticotrophic activity stimulates steroid production through activation of the adenylate cyclase system of the adrenocortical cells [4]. The reduction in the content of cAMP in the thymus after 300 min of CST could indicate the beginning of depression of the functions of the lymphoid-macrophagal system, which is a feature of severe stress.

Depression of activity of the thyroid complex in stress is a well known fact [5]. One of the causes of this depression, in particular, could be the observed increase in adrenocortical activity, for glucocorticoids directly or indirectly can inhibit thyroid gland function [10].

The effect of preliminary injection of enkephalin was manifested after 300 min of CST as a fall in the plasma cortisol level and cAMP concentration in the adrenals compared with the control, elevation of the cAMP level in the thymus, and higher values of parameters of pituitary-thyroid functions.

In previous investigations, during stress induced by acute myocardial ischemia, a similar action of enkephalin was demonstrated on the dynamics of glucocorticoid activity at these same times [2]. The enkephalin now being tested evidently has a suppressive action on function of the hypothalamo-hypophyseo-adrenal system (HHAS). Proof of this is given by the fall in the ACTH level after 60 min of CST, when the enkephalin was used, and in the plasma cortisol concentration and the cAMP concentration in the adrenals after 300 min of CST, compared with the control. This action of enkephalin may probably be aimed at preventing exhaustion of the adrenal cortex in severe stress.

The property of enkephalin of preventing stress-induced inhibition of activity of the pituitary-thyroid complex, and also its ability to increase functional activity of thymus cells through an increase in cAMP, can evidently be interpreted as a positive fact. Its antiadrenergic properties may also play a role in the mechanisms of the antistressor effects of enkephalin [6].

An important role in the mechanism of the antistressor protective effects of enkephalin is thus evidently played by its ability to prevent inappropriate exhausting hyperfunction of the HHAS, and also to prevent stress-induced inhibition of the pituitary-thyroid complex and of the lymphoid-macrophagal system.

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EFFECT OF α-TOCOPHERYL ACETATE ON SOME BLOOD BIOCHEMICAL PARAMETERS OF ALBINO RATS EXPOSED TO ACOUSTIC STRESS

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KEY WORDS: noise; lipid peroxidation; α-tocopherol; cholesterol.

There is much evidence in the literature on the harmful action of sound on the body [11, 12]. An important role in the mechanisms of realization of the effects of various pathological factors has recently been ascribed to changes in the intensity of free-radical processes taking place in the body. Marked intensification of lipid peroxidation (LPO) has been demonstrated in various extremal states [3, 5]. In view of the facts mentioned above, and also of evidence [4] of the direct formation of free radicals in an aqueous medium under the influence of mechanical oscillations with a frequency of 7-200 Hz in experiments *in vitro*, in order to determine the degree of participation of LPO in the mechanisms of realization of the pathological effects of noise on the body the effectiveness of induction of LPO was studied in various tissues of albino rats [6, 7] and considerable changes were demonstrated in the LPO system in the brain, liver, and heart. This paper gives the results of an investigation of the intensity of LPO and also of the level of the endogenous antioxidant α-tocopherol, and of the risk factor, cholesterol, in the plasma and erythrocyte membranes, in animals receiving α-tocopheryl acetate and exposed to the action of noise.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-220 g, kept under ordinary animal house conditions. The animals were divided into six groups: rats of group 1 served as the control, rats of groups 2-6 were exposed to the action of noise (91 dB) with maximal energy in the region of middle and high frequencies. Additionally, in each experimental group a subgroup of rats receiving α-tocopheryl acetate (TPA) intraperitoneally throughout the experiment in a dose of 1 mg/kg was distinguished. The duration of exposure to noise of animals of the various groups was 1 and 8 h, and 7, 28, and 56 days, respectively, for 8 h each day.

The animals were decapitated. The background level of lipid peroxides (LP) in the plasma was determined by the method in [14]. The LP concentration was expressed in nmoles malonic dialdehyde (MDA) per 1 ml of plasma. Erythrocyte membranes were isolated by the method in [15]. Activity of LPO systems in erythrocyte membranes was determined by measuring accumula-

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