CHANGES IN EOSINOPHIL AND CORTICOSTERONE LEVELS AND CATECHOLAMINE METABOLISM DURING EMOTIONAL-PAINFUL STRESS

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UDC 613.863-07:[616.155.35+616.453-008.6+616.452-008.6

KEY WORDS: emotional-painful stress; eosinophils, corticosterone; catecholamines.

Exposure to various kinds of stress causes changes of phasic character in the peripheral blood eosinophil count [6, 7]. After the end of exposure to stress eosinopenia develops and, after a certain length of time, changes into transient eosinophilia. Fluctuations in the eosinophil count subsequently gradually reduce toward the original biorhythm. During eosinopenia changes in metabolism and in the structure and function of the heart reach their maximum [4], and the time of onset of eosinophilia may be used as an indicator of the intensity of the stress factor or the level of resistance of the organism [5]. However, the relations between the time course of the blood eosinophil count and of catecholamine and glucocorticoid metabolism in the regulatory system and effector organs after exposure to stress have not yet been elucidated.

The aim of this investigation was to study blood levels of eosinophils, corticosterone (CS), adrenalin, noradrenalin (NA), and dopamine (DA) during development of the stress reaction, and also to study neuronal uptake and synthesis of catecholamines in the adrenals and heart.

EXPERIMENTAL METHOD

Experiments were carried out on 172 male albino rats weighing 200-220 g with an initial peripheral blood eosinophil count of 220-340/μl at 9 a.m. Emotional-painful stress (EPS) was induced for 6 h in the form of an anxiety neurosis by the method in [13]. The experimental animals as a whole were divided into four main groups. In the animals of group 1 eosinophils were counted in 1 μl blood taken from the caudal vein every 3 h after the end of induction of EPS for 216 h. Eosinophils were stained by Hinkleman's method and counted in a Goryaev's chamber. Animals of group 2 were decapitated immediately and also 2, 24, 36, 45, 54, 72, 96, and 216 h after the end of exposure to stress, and the CS concentration was determined in blood plasma, the heart, and adrenals. The hormone was extracted with methylene chloride and subjected to chromatography on columns with silica gel [8]. At the same time, and in these same animals, concentrations of adrenalin, NA, and DA in the heart and adrenals were determined fluorometrically by the trihydroxyindole method [9]. In animals of groups 3 and 4 the neuronal uptake of ^{3}H-NA and the intensity of ^{3}H-Na and ^{3}H-DA synthesis from ^{3}H-tyrosine were investigated by the method described previously [10] 2 h after the end of induction of EPS (in isolated atria and adrenals). The following radioactive preparations were used: ^{3}H-NA from Amersham Corporation, England and ^{3}H-tyrosine from Izotop, USSR. Radioactivity was measured on an SL-30 liquid scintillation counter (Intertechnique, France). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After the end of induction of EPS the time course of the blood eosinophil level revealed three principal periods with different eosinophil counts (Fig. 1, I): the first period—eosinopenia (0-39 h), the second — eosinophilia (39-45 h), and the third period, when after a second short period of eosinopenia fluctuations in the blood eosinophil level gradually dimished toward the original biorhythm (45-216 H).

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(Presented by Academician of the Academy of Medical Sciences of the USSR K. R. Sedov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 267-269, March, 1985. Original article submitted March 28, 1984.

0007-4888/85/9903-0249$09.50

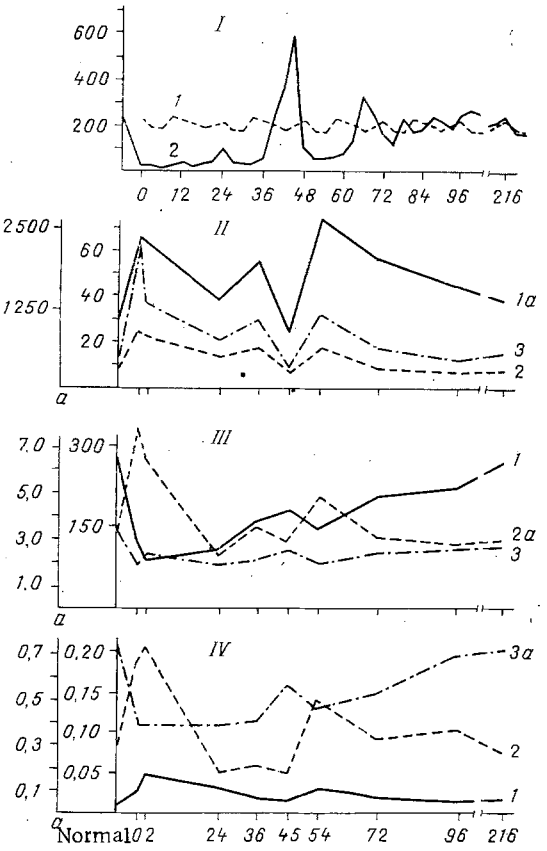

Fig. 1. Changes in eosinophil, CS, and catecholamine levels during EPS. Abscissa, time after end of induction of EPS (in h); ordinate, number of eosinophils in 1 µl blood, concentrations of CS and catecholamines. I) eosinophil count in peripheral blood: 1) control, 2) EPS; II) CS concentration (in µg%): 1a) in adrenals, 2) in plasma, 3) in heart; III) catecholamine concentration in adrenals (in µg/g): 1) adrenalin, 2a) DA, 3) NA; IV) catecholamine concentration in heart (in µg/g): 1) adrenalin, 2) DA, 3a) NA. At each time indicated 12 animals were tested.

TABLE 1. Neuronal Uptake of ^{3}H-NA (I) and Synthesis of ^{3}H-NA (II) and ^{3}H-DA (III) from ^{3}H-tyrosine in Atria and Adrenals of Rats with EPS (cpm/g; M ± m)

Organ	Exptl. conditions	Control	EPS
Atrium	I	70 654±4 465	48 581±3 518***
	II	7 363±510	5 241±578*
	III	1 903±75	2 166±264
Adrenal	I	53 850±2 707	41 182±3 868*
	II	7 158±604	5 294±228**
	III	4 250±321	4 304±539

Legend. *P < 0.05; **P < 0.01; ***P < 0.001; 12 animals used in each series.

Analysis of the catecholamine and CS levels showed that the period of eosinopenia corresponded to marked activation of the adrenergic and pituitary-adrenal systems (Fig. 1, II-IV). For instance, immediatley after exposure to stress and for the next 36 h the CS concentration in the plasma, heart, and adrenals rose by 2.5-3 times. At the same time the adrenalin concentration increased and the NA level diminished by half in the heart. The adrenalin concentration in the adrenals fell to 40-60% of normal. The DA concentration in the organs studied increased by 2-2.5 times under these circumstances. The results are evidence that the eosinopenic effect in stress is due to a rise in the concentration of glucocorti-

coids, which cause a redistribution of eosinophils from the blood into the organs, their storage in the depots, and inhibition of eosinophil production in the bone marrow [1, 2]. Data in Table 1 shed some light on the principal mechanisms of disturbance of catocholamine metabolism in EPS. It will be clear from Table 1 that the fall in the NA level in the heart and adrenalin level in the adrenals was due to marked inhibition of neuronal uptake and synthesis of these monoamines. The decrease in activity of ^{3}H-NA synthesis during EPS may be associated with exhaustion of the enzyme systems [11] or inhibition of tyrosine hydroxylase by high DA concentrations in the organs tested [3]. One cause of the decrease in intensity of neuronal uptake may evidently be the action of high concentrations of glucocorticoids [12]. The increase in DA concentration in the heart and adrenals, against the background of no change in the intensity of ^{3}H-DA synthesis from ^{3}H-tyrosine in these organs can be regarded as the result of inhibition of dopamine-β-oxidase, which likewise may limit the synthesis of NA and cause its level in the organ to fall. This possibility is indicated by the results of experiments with dopamine-β-oxidase inhibitors [14, 15]. The authors cited showed that administration of disulfiram or picolinic acid derivatives to animals (against the background of induction of stress) causes a sharper fall of the NA concentration in the heart.

During the period of eosinophilia a sharp decrease in the CS level was observed: to normal in the plasma, to 54 and 83% of the control respectively in the heart and adrenals. The NA concentration in the heart during this period fell by 26% and DA by 37%. The adrenalin concentration in the adrenals was 72% of its initial value.

In our view the appearance of eosinophilia after eosinopenia 45 h after the end of exposure to stress was due to a decrease in the CS concentration in this period, leading to expulsion of eosinophils from the bone marrow and organs into the blood stream [2]. In this period, during extinction of the stress reaction, the synthesis and neuronal uptake of catecholamines are evidently activated, as is shown by the relative rise in concentrations of NA in the heart and adrenalin in the adrenals, and also a fall in the DA concentration.

During the third period of stress-induced changes in blood eosinophil levels (51-57 h after exposure), the temporary eosinopenic effect was accompanied by reactivation of the adrenergic and pituitary-adrenal systems. In our view this increase in activity of the stress-realizing systems was connected with the formation of maximal disturbances of cardiac metabolism, structure, and function [4] in the preceding period, which could be an endogenous cause [16] of reappearance of a transient stressor reaction. Later (on the 8th-9th day after induction of EPS) the CS and catecholamine concentrations gradually approached their initial level, and this corresponded in time with normalization of the blood eosinophil biorhythm.

A regular relationship was thus found between changes in the blood eosinophil level, the CH concentration, and catecholamine metabolism in the course of EPS. Differences discovered in function of the adrenergic and pituitary-adrenal systems reflect adaptive changes in biorhythms of neuroendocrine control of homeostasis and provide a basis for the objective determination of parameters of the periods of formation of the stress reaction during exposure to various extremal influences.

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ENKEPHALINS AND HORMONAL-METABOLIC REACTIONS IN EXPERIMENTAL STRESS DEPENDING ON ITS SEVERITY

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UDC 613.863-07:[616.634.452+616.154.37+616.154.453]-02:615.31:[547.95:547.943

KEY WORDS: enkephalins; stress; hormones; catecholamines.

Elevation of the blood level of endogenous opioid peptides during stress is a sufficiently well documented fact [7, 8]. However, the physiological significance of this phenomenon is not yet clear. Previously the writers showed that enkephalins promoted normalization of several metaboblic parameters (water-electrolyte and acid-base balance, the blood enzyme spectrum) and reduced the mortality of animals with experimental myocardial infarction [2, 3]. The hyperergic response of the principal hormonal stress systems was prevented under these circumstances [4]. The beneficial action of enkephalins on the course of myocardial ischemia may perhaps be associated with the alleviation of stress-induced injuries.

The aim of this investigation was to study the action of enkephalins on changes in hormonal-metabolic constants in stress of varied severity.

EXPERIMENTAL METHOD

Experiments were carried out on 135 male albino rats weighing 160-180 g, divided into groups with 8-12 animals in each group. The models of stress used were suspending the animals by the fold of the neck for 3.5 h, deprivation of food for 72 h, a combination of these procedures, and acute myocardial ischemia [9]. In each version of the experiments half of the animals were treated by daily injections of the stable arginine-containing hexapeptide Leu-enkephalin analog (LE), obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director, Dr. Chem. Sci. M. I. Titov), in a dose of 1.25 nmole/100 g body weight intraperitoneally. The remaining animals were given physiological saline in equivalent volumes. The state of stress was evaluated by measuring the absolute blood concentrations of glucocorticoids and insulin and determining a coefficient reflecting relative percentages of these hormones [6]. Catecholamine excretion with the urine was determined fluorometrically on a "Hitachi" (Japan) spectrofluorometer, serum cortisol and insulin concentrations were measured radioimmunologically, using standard kits from "CEA-Sorin" (France) on a "Tracor" gamma-spectrometer (USA), and glucose was determined by the standard orthotoluidine method.

EXPERIMENTAL RESULTS

Suspending the rats by the fold of the neck for 3.5 h caused no change in the immunoreactive cortisol level or insulin activity in the blood (Table 1). A small increase in the cortisol/insulin ratio (C/I) was observed. It can accordingly be concluded that this ver-

Laboratory of Pathophysiology, Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Tomsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 269-271, March, 1985. Original article submitted June 11, 1984.

 0007-4888/85/9903-0252$09.50