LOWERING PLASMA IMMUNOREACTIVE α- AND γ-ENDORPHIN LEVELS AND INHIBITION OF THEIR HYPERSECRETION BY DEXAMETHASONE IN MONKEYS WITH EMOTIONAL STRESS

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The endorphins are a group of peptides of opioid nature which, together with adrenocorticotrophic hormone (ACTH), α-, β-, and γ-melanocyte-stimulating hormones (MSH), and β-lipotrophin, are formed from the same high-molecular-weight precursor protein — proopiomelanocortin (POMC) [2, 3, 5, 10]. α-, β-, and γ-Endorphins are distinguished, and are fragments 61-77, 61-91, and 61-76 respectively of β-lipotrophin [2, 3, 5]. The sources of the β-endorphin tested in the blood are the anterior and intermediate lobes of the pituitary gland [2, 3, 5]. Synthesis of POMC in the anterior pituitary is inhibited by glucocorticoids [2, 5, 6, 8]; under these circumstances the blood levels of ACTH, α-MSH, and β-endorphin fall [2, 5, 7, 9]. In stress the blood levels of ACTH and β-endorphin are raised and both peptides are secreted in equimolar amounts [2, 5, 11].

We studied the action of long-term injections of dexamethasone and of acute emotional stress on blood levels of α- and γ-endorphins in the baboon *Papio hamadryas*.

EXPERIMENTAL METHOD

The following reagents were used: bovine serum albumin, EDTA, Na_2HPO_4, NaH_2PO_4, NaCl (from Sigma, USA), polyethylene-glycol (6 kD) (from Merck, West Germany), and dexamethansone from Galenika (Yugoslavia). Endorphins were synthesized and generously provided by Professor M. I. Titov (All-Union Cardiologic Center, Moscow).

Experiments were carried out on 14 mature male baboons aged 8-9 years and weighing 27-31 kg. The experimental animals were subjected to immobilization for 2 h in three groups: group 1 (control) received physiological saline daily for 10 days, group 2 received 4 mg of dexamethasone on a similar schedule, and the animals of group 3 received dexamethasone in a daily dose increased to 12 mg. Immobilization of the animals involved fixing them horizontally in the supine position with the head and limbs secured, for 2 h in the morning (10 a.m.-12 noon) before taking food. Blood for endorphin and cortisol assay was taken at 10 a.m. before a meal, immediately before injection of the saline or dexamethasone, and thereafter on the 4th, 7th, and 10th days from the beginning of the experiment. In the experiments with immobilization stress blood was taken immediately before the beginning of the experiment and thereafter 2, 6, 24, 48, and 72 h after the beginning of immobilization. Blood (10 ml in a tube containing 0.15 ml of 0.5 M EDTA) was taken from the cubital vein, mixed, cooled to 0°C, centrifuged in the cold (1000g, 15 min), and the plasma kept at −70°C.

α- and γ-Endorphins were determined in the plasma without preliminary extraction as described previously [4]. The antisera used for radioimmunoassay were characterized by the following specificity: antiserum No. 80 to α-endorphin gave a crossed reaction with β-endorphin 2.6%, γ-endorphin of 14.0%, and with bovine β-lipotrophin of 0.3%; antiserum No. 49 to γ-endorphin gave a crossed reaction with α-endorphin of 0.3%, with β-endorphin of 4.4%, and with

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TABLE 1. Plasma Levels of Cortisol and α- and γ-Endorphins in Male Baboons (*P. hamadryas*) Receiving Daily Injections of Dexamethasone for 10 Days, Followed by Immobilization for 2 h (M ± m)

Substance injected	Compound determined	Time after beginning of injection of subs., days				Time from beginning of immobilization for 2 h,				
		0	4	7	10	2	6	24	48	72
Physiological saline (n = 5)	Cortisol, ng/ml	253±30	241±16	—	309±30	447±44*	571±17*	304±17	335±6	310±22
	α-Endorphin, pM	316±13	313±26	324±27	362±40	731±70*	992±38*	476±47	337±33	284±24
	γ-Endorphin, pM	20,7±0,9	18,7±0,5	20,0±1,1	21,2±1,4	39,9±3,7*	44,2±3,1*	28,7±4,3	25,0±2,3	18,7±1,3
Dexamethasone, 4 mg daily (n=5)	Cortisol, ng/ml	290±16	269±23	253±24	262±26	353±17*	394±29*	318±29	296±23	300±9
	α-Endorphin, pM	312±13	335±44	294±29	318±36	417±39*	612±97*	384±35	159±12	252±12
	γ-Endorphin, pM	20,1±1,2	21,1±1,2	19,7±1,1	16,0±1,8	22,8±0,6	26,1±1,8*	18,3±1,5	11,3±2,3	21,3±1,7
Dexamethasone, 12 mg daily (n=4)	Cortisol, ng/ml	280±13	66±14*	41±17*	36±8*	124±30*	70±17*	38±9	170±38	239±28
	α-Endorphin, pM	300±10	194±13*	172±10*	167±16*	200±26	215±22	157±20	149±4	90±9
	γ-Endorphin, pM	22,3±1,8	18,1±0,7	13,6±1,4*	12,3±0,2*	17,7±1,5	23,8±1,1*	16,3±2,1	15,0±16,9	14,7±3,0

Legend. Significance of differences (*) for injections of physiological saline or dexamethasone indicated relative to concentration observed before beginning of injections; in immobilization stress, significance of differences determined relative to concentration present on the 10th day of injection of corresponding substance.

bovine β-lipotrophin of 0.5%. The sensitivity of radioimmunoassay of γ-endorphin was 25 fmoles/ml and of γ-endorphin 5 fmoles/ml. Cortisol was determined by means of kits from "Daiichi" (Japan). The data were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The effect of long-term action of dexamethasone on concentrations of α- and γ-endorphins and of cortisol in the blood was studied in male baboons. In the group of baboons receiving 4 mg dexamethasone daily, just as in the control, no change was observed in the plasma cortisol concentration and the concentrations of α- and γ-endorphins remained at the previous level. With an increase in the daily dose of dexamethasone to 12 mg per animal, the plasma cortisol concentration fell significantly by the 4th day of the injections (Table 1). The α-endorphin level also fell to 194 ± 13 pM, 35% lower than initially ($p < 0.05$). The γ-endorphin concentration fell in a similar manner, but the fall was significant only on the 7th day after the beginning of the injections (1.7 times less, $p < 0.05$). On the 10th day after the beginning of injections the γ-endorphin level was 12.3 ± 0.2 pM, or 1.8 times ($p < 0.01$) less than initially (Table 1).

In the next series of experiments the effect of immobilization stress was studied on blood levels of α- and γ-endorphins and cortisol in the baboons. After immobilization for 2 h, the experimental animals of all three groups showed a marked increase in the cortisol concentration, which reached a maximum after 6 h. Later the value of this parameter fell (Table 1). A similar tendency was observed for α- and γ-endorphins in the control group and in the group of monkeys receiving 4 mg dexamethasone daily. In the control group, plasma α- and γ-endorphin concentrations increased significantly (by twice for each peptide) 2 h after the beginning of immobilization, and reached a maximum 6 h after the beginning of immobilization (by 2.7 times for α-endorphin and by 2.1 times for the γ-peptide; in both cases $p < 0.01$). The plasma endorphin levels returned to normal after 24 h. In the group of monkeys receiving 4 mg dexamethasone daily for 10 days a similar picture was observed, but the increase in the endorphin concentration during stress was somewhat less marked. For instance, the α-endorphin concentration was raised by 1.9 times and the γ-endorphin by 1.6 times; the maximal increase also was observed 6 h after the beginning of immobilization (Table 1). In the group of animals receiving dexamethasone in a dose of 12 mg daily for 10 days, significant inhibition of α-endorphin secretion was observed during immobilization stress for 2 h. The blood level of this peptide was raised by only 1.28 times (6 h after the beginning of immobilization). Under the same conditions the γ-endorphin concentration rose by 1.9 times, but in this case levels of both α- and γ-endorphins were 2-3 times lower than in the control group 6 h after the beginning of immobilization stress (Table 1). The initial blood levels of α- and γ-endorphins were restored on the 2nd-3rd days after the beginning of stress.

The pool of POMC fragments detectable in blood is considered to be due to virtually independent secretion of the anterior and intermediate lobes of the pituitary gland [2, 5]. Glucocorticoids act on synthesis and secretion of POMC fragments formed only in the anterior lobe of the pituitary [2, 5, 6, 8]. Hypersecretion of ACTH and β-endorphin, observed during stress, evidently is also brought about by the anterior lobe of the pituitary [2, 5]. Long-term injections of high doses of dexamethasone lower the blood concentrations of α- and γ-endorphins by half and depress the hypersecretion of these peptides in acute emotional stress.

Glucocorticoids depress synthesis and secretion of α- and γ-endorphins by cells of the anterior lobe of the pituitary. Synthesis of these peptides in the intermediate lobe of the pituitary is evidently unaffected. The action of glucocorticoids on the blood α- and γ-endorphin levels suggests that as well as ACTH, β-endorphin, and α-MSH [13], a change in the concentration of these peptides may act as markers of Cushing's and Addison's diseases.

In baboons with acute emotional stress hypersecretion of α- and γ-endorphins is observed. It was demonstrated previously that in a stress state connected with the presence of acute bronchial asthma, a simultaneous rise of the plasma α-, β-, and γ-endorphin levels was observed [1]. In stress the blood concentration of other POMC fragments is increased: ACTH, γ-MSH, and β-endorphin [2, 5, 11, 12]. Thus an increase in the blood α- and γ-endorphin concentrations can also serve as an indicator of a stress state.

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CONCENTRATIONS OF CYCLIC NUCLEOTIDES IN ORGAN CULTURES OF NORMAL AND ATHEROSCLEROTIC HUMAN AORTA

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Disturbances of function of the cyclic nucleotide system are observed in various pathological states [5]. In particular, it has been shown that in experimental atherosclerosis the content of cyclic AMP and cyclic GMP in atherosclerotic arteries differs significantly from their levels in arteries of control animals [7-9]. However, there are no data in the literature on cyclic nucleotide levels in the human aorta in spontaneous atherosclerosis. In the present investigation, a short-living organ tissue culture was used to study cAMP and cGMP concentrations in atherosclerotic and unaffected areas of the human aorta.

EXPERIMENTAL METHOD

Autopsy material was taken from unhospitalized men aged 40-60 years dying suddenly from myocardial infarction, 0.5-1.5 h after death. After removal of the adventitia the aorta was washed with isotonic phosphate buffer (IPB) and tissue fragments were excised (1 × 1 cm) from three different areas: with no signs of atherosclerosis, from lipid streaks, and from atherosclerotic plaques. The intima and media were separated mechanically and each layer cultured separately in a dish 35 mm in diameter containing 2 ml of medium 199 (Gibeo, England) at a temperature of 37°C in an atmosphere of CO_2 and air in the ratio of 5:95, satured with water. At the end of incubation, tissue fragments were quickly washed with IPB solution and frozen in liquid nitrogen. Cyclic nucleotides were extracted by grinding the frozen tissue in a mortar with 2 ml of 96% ethanol. To estimate losses, 0.05 pmole of [^{3}H]cAMP (Amersham International, England) was added to the homogenate. The resulting homogenate was centrifuged (2500g, 30 min) and the residue washed twice in 4 ml of a mixture of 96% ethanol and water in the ratio of 7:1 (v/v). DNA was extracted from the residue [10] and assayed by Burton's method [4]. The pooled supernatant was applied to a column (0.6 × 2 cm) containing 0.5 g of dry alumina (Merck, West Germany). Nucleosides and purine bases were removed by washing the column with 6 ml of a mixture of ethanol and water (7:1, v/v), and the cyclic nucleotides were eluted with 2 ml of water-ethanol mixture (2:1, v/v). The eluate was evaporated at a temperature of 70°C under reduced pressure and dissolved

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