EFFECT OF DALARGIN ON BLOOD ENDORPHIN, LEU-ENKEPHALIN, ACTH, AND CORTICOSTERONE LEVELS IN STRESSED RATS

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In previous publications the writers presented information on compensated and decompensated manifestations of stress at the regulation and metabolism level [7] and, in particular, on disturbances of distribution of corticosterone in the blood, liver, and heart of rats, disturbances of glucocorticoid receptor binding, and dramatic fluctuations in the metabolic profile in the liver, with the possibility of severe exhaustion of the energy potential of the cell in the terminal stage.

In this investigation the effect of dalargin, a synthetic enkephalin analog with glycine replaced by D-Ala^2, and known for its antiulcerative action, was studied on blood levels of endorphins, Leu-enkephalin, ACTH, and corticosterone of stressed rats.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 100-150 g, in which acute peritonitis was induced. For this purpose the rats were given an intraperitoneal injection of various doses of a freshly prepared 5% suspension of feces in physiological saline. The first toxic manifestations appeared in the animals after only 1.5-2 h. At autopsy, irrespective of the time elapsing after injection of the fecal suspension and the dose, changes of varied severity were discovered in the abdomen, ranging from the appearance of a moderate quantity of peritoneal exudate to a very abundant sero-hemorrhagic effusion with deposition of fibrin, severe edema of the liver, spleen, and kidneys, necrosis of single loops of intestine, and foci of necrosis in the liver and spleen. Animals of groups 1 and 2 (45 rats in each group) were given an injection of 0.5 ml of a 5% suspension of the toxin, whereas animals of groups 3 and 4, also with 45 per group, were given 1 ml of the same suspension. Rats of groups 2 and 4 received an intramuscular injection of dalargin in physiological saline at the same time in a dose of 10 µg/kg body weight, and the same dose also was repeated 16 h later. The blood levels of endorphins, Leu-enkephalin, ACTH, and corticosterone were studied in rats of all groups (n = 15 in each group at the beginning of the experiment) 2, 16, and 24 h after injection of the suspension of toxin. The control consisted of 25 intact animals.

EXPERIMENTAL RESULTS

When 0.5 ml of a 5% suspension of toxin was injected (experiments of series I) the following trend of the parameters studied was discovered in the rats' blood (Table 1). A significant increase in concentrations of β-endorphin, Leu-enkephalin, ACTH, and corticosterone was found in these animals 2 h after injection of the toxic suspension compared with intact rats. After 16 h the β-endorphin level continued to rise, but the Leu-enkephalin level fell sharply and the ACTH and corticosterone concentrations also decreased (the corticosterone level was nevertheless 2.5 times higher than in intact animals). Normal blood levels of β-endorphin and corticosterone were discovered 24 h after the beginning of the experiment, together with a low concentration of Leu-enkephalin and, in particular, of ACTH.

A link with the phase of the stress reaction could be clearly identified in the action of dalargin on the trend of the parameters studied during its courses. For instance, in the first half of the 24-h period (from 2 a.m. to 4 p.m.) the raised Leu-enkephalin, ACTH, and β-endorphin levels were significantly reduced by the action of dalargin, whereas in the second half of the 24-h period (from 4 p.m. until midnight), on the contrary, against the background of normal or low values of these parameters a significant increase was observed, under

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TABLE 1. Effect of Dalargin on Blood Levels of β-Endorphin, Leu-Enkephalin, ACTH, and Corticosterone in Rats After Intraperitoneal Injection of 0.5 ml of 5% Toxic Suspension (M ± m)

Parameter studied	Control (intact rats)	Experiment (acute peritonitis)					
		After 2 h		After 16 h		After 24 h	
		Without dalargin	With dalargin	Without dalargin	With dalargin	Without dalargin	With dalargin
β-endorphin, pmoles/liter	17,90±0,17	27,00±0,12[a]	27,00±0,09[a]	56,40±0,35[a]	40,20±0,26[a,b]	17,80±0,16	39,60±0,39
Leu-enkephalin, pg/ml	1251±95	2376±94[a]	1731±100[a,b]	998±31[a]	1304±93[b]	807±29[a]	910±50[a]
ACTH, pg/ml	209,0±17,2	336,0±45,6[a]	238,0±5,8	152,0±10,7[a]	146,0±13,5[a]	84,8±5,5[a]	149,0±15,5[a,b]
Corticosterone, ng/ml	114,0±6,2	550,0±17,4[a]	559,0±5,2[a]	252,0±12,2[a]	276,0±1,7[a]	108,0±1,0	215,0±19,2[a,b]

Legend. Here and in Table 2: a) $P < 0.005$ compared control, b < 0.05 on comparison of groups of animals receiving dalargin.

TABLE 2. Effect of Dalargin on Blood Levels of β-Endorphin, α-Endorphin, γ-Endorphin, Leu-Enkephalin, ACTH, and Corticosterone in Rats Receiving Intraperitoneal Injection of 1 ml of 5% Toxic Suspension (M ± m)

Parameter studied	Control (intact rats)	Experiment (acute peritonitis)					
		After 2 h		After 16 h		After 24 h	
		Without dalargin	With dalargin	Without dalargin	With dalargin	Without dalargin	With dalargin
β-endorphin, p moles/liter	22,80±1,26	36,10±2,95[a]	25,30±2,61b	52,10±4,07[a]	43,30±1,64[a]	30,70±1,03[a]	19,10±0,94[6]
α-endorphin pg/ml	346,0±15,2	853,0±92,0[a]	420,0±44,7[b]	331,0±29,8	398,0±38,9	259,0±11,5[a]	782,0±35,3[a,b]
γ-endorphin pg/ml	30,40±0,42	34,70±1,41	34,50±0,59	31,80±0,64	41,60±2,08[a,b]	33,90±1,16	61,30±1,54[a,b]
Leu-enkephalin, pg/ml	1564±176	3082±70[a]	1647±216	553±34[a]	1003±50[a,b]	1085±13[a]	2437±60[a,b]
ACTH, pg/ml	532,0±35,8	458,0±62,5	364,0±10,0[a]	721,0±40,6[a]	558,0±8,3[b]	634,0±30,3	595,0±27,6
Corticosterone, ng/ml	196,0±9,5	560,0±23,0[a]	536,0±15,1[a]	615,0±33,9[a]	509,0±17,1[a,]	241,0±6,2[a]	241,0±25,8

the influence of dalargin, in the blood levels of β-endorphin, ACTH, and corticosterone.

After injection of 1 ml of the 5% toxic suspension into the rats (experiments of series II) the trend of the blood levels of β-endorphin, Leu-enkephalin, ACTH, and corticosterone as a whole was indistinguishable from that in the experiments of series I. The difference was (Table 2) that the ACTH level 2 after the beginning of the experiment was already lowered (by a feedback mechanism), whereas the corticosterone level was still continuing to rise after 16 h, evidence of a stronger stress reaction than in the experiments of series I (Table 1). Concentrations of α- and γ-endorphin were additionally determined in the blood. Elevation of the α-endorphin level was discovered 2 h after the beginning of the experiment, followed by a fall. The blood γ-endorphin level remained unchanged in the course of the stress reaction. The action of dalargin confirmed the data obtained in the experiments of series I: under the influence of the drug there was a significant fall of the raised blood levels of β-endorphin, α-endorphin, and Leu-enkephalin (during the first half of the 24-h period) and a rise of the lowered or normal Leu-enkephalin and endorphin levels.

The absence of any distinct direct action of dalargin on the blood corticosterone level (according to the results of the two series of experiments) is noteworthy. The change in the blood corticosterone concentration in the course of stress is linked more closely with changes in the β-endorphin, Leu-enkephalin, and ACTH levels under the influence of dalargin.

These results are evidence that all detectable types of endogenous opioids (except γ-endorphin) are involved in the mechanism of the stress reaction. The opposite changes in endorphin and Leu-enkephalin levels in stress are evidently due not only to changes in ACTH secretion, but also to correction of the glucocorticoid activity of the adrenals. As regards the role of individual opioids, the antistressor role of Leu-enkephalin seems to be more definite, for its time course during stress is similar to that of ACTH. Changes in the blood endorphin levels, on the other hand, were similar to the time course of the corticosterone concentration. Indirect confirmation of this hypothesis can be found elsewhere [10, 12, 14].

More marked stress (Table 2) is accompanied by disturbance of relations between ACTH and corticosterone: the ACTH level falls rapidly by a feedback mechanism, but this is not followed by a fall in the blood corticosterone level (a distinctive loss of control of adrenal glucocorticoid activity by ACTH for a considerable length of time — up to 16 h). Nevertheless, attention is drawn to the compensated character of the hormonal deviations.

The action of dalargin, administered to rats with the aim of protection, has a normalizing tendency in stress in relation to endorphin, Leu-enkephalin, and ACTH concentrations.

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