NORMALIZING EFFECT OF DALARGIN ON BLOOD GLUCOCORTICOID AND OPIOID LEVELS IN CBA AND C57BL/6 MICE UNDER FOOTSHOCK STRESS CONDITIONS

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Relations between changes observed in metabolism of different groups of endogenous opioids under stress conditions vary considerably depending on the characteristics of the stressor concerned [8]. Meanwhile, about 30 endogenous ligands of opioid receptors (OR) are now known [2, 7]. It is therefore essential to study integral characteristics of the state of the endogenous opioid system during stress and correlation between these changes and the characteristics of steroid metabolism.

As a result of experimental and clinical investigations it has also been shown that agonists of OR of the δ-type can exert a stress-limiting effect on the state of the body [2, 3, 5]. However, the action of opioids of this group on total activity of ligands of the basic types of OR has not been studied during development of the stress reaction. The possible dependence of the action of OR agonists of the δ-type on the state of the body likewise have not been adequately studied, and the duration of the effects discussed is not clear.

Accordingly, the investigation described below was carried out with two main aims: to assess the dynamics of changes in activity of ligands of OR of the μ- and δ-types and plasma steroid hormone levels in inbred mice after footshock stress and to study the effect of dalargin, a synthetic agonist of OR of the δ-type, on these parameters.

EXPERIMENTAL METHOD

Male CBA and C57BL/6 mice (18-20 g), kept in the animal house under standard conditions of lighting, temperature, and food, were used. The period of acclimatization of the animals before the investigation began was not less than two weeks.

For the model of footshock stress we used chambers manufactured by the "Diagnostika" Research-Engineering Combine. An electric current was applied to the floor of the chamber in accordance with the following schedule: [(1.5 mA × 5 sec, interruption for 5 sec) × 25 times, 30 sec with interruption] × 3 times – total duration of procedure 13 min.

Dalargin (5 μg/kg) in 0.5 ml physiological saline in the experimental group or 0.5 ml physiological saline in the control group was injected into the orbital sinus 5 min after the end of the session of electrical stimulation.

The animals were killed by decapitation 20 min, and 1, 3, 10, and 24 h after the end of stress. Blood was collected, using a 3% solution of the sodium salt of EDTA (in the ratio of 1:10) as the anticoagulant. To prevent proteolysis of the endogenous peptides, the protease inhibitor bacitracin (200 μg/ml blood) was added. The blood was centrifuged (1000*g*, 10 min, 4°C). The plasma was collected, frozen, and kept at −70°C.

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TABLE 1. Action of Dalargin on Level of Displacing Activity of OR Ligands in Blood Plasma of Mice After Footshock Stress (M ± m, n = 11)

Experimental series		Time after procedure				
		20 min	1 h	3h	10h	24h
		pmole-equivalents DAGO/ml plasma				
CBA	Physiological saline (PS)	161±7**	120±4	97±8	134±6**	118±6
	Dalargin	112±5*	96±4*	102±6	97±5*	109±6
C57Bl/6	PS	123±6**	125±7**	104±9	96±9	107±8
	Dalargin	95±4*	99±15	96±4	103±4	113±7
		pmole-equivalents DADLE/ml plasma				
CBA	PS	254±6	198±10**	176±13**	273±6**	314±15**
	Dalargin	207±12*	209±11	241±28	210±13*	256±10*
C57Bl/6	PS	179±16	210±24	213±18**	252±10**	264±25**
	Dalargin	158±10	180±12	153±10*	167±9*	249±19**

Legend: *$p < 0.05$ compared with animals receiving physiological saline; **$p < 0.05$ compared with intact animals. Displacing activity of OR ligands in 1 ml plasma of intact animals was 99 ± 5 and 248 ± 7 pmole-equivalents DAGO and DADLE respectively in CBA mice; corresponding values for C57BL/6 mice were 95 ± 6 and 145 ± 5 pmole-equivalents DAGO and DADLE.

To obtain the extract, the plasma was thawed and transferred into 0.25 M acetic acid (ratio 1:9). The resulting solution was frozen, lyophtlized, and kept at −70°C. Immediately before radioreceptor analysis (RRA) the samples were dissolved in 50 mM Tris-HCl buffer (pH 7.7, at 22°C), centrifuged (8000*g*, 15 min, 22°C), and then used in RRA.

The membrane fraction of the Wistar rat brain for RRA was obtained and activity of the plasma extracts for displacement of ligands from OR determined as we described previously [4]. As labeled ligands we used ^{3}H-D-Ala^2-MePhe^4-Gly(ol)^5-enkephalin (^{3}H-RX 783006 or ^{3}H-DAGO), the ligand of OR of the μ-type, in a concentration of 40 Ci/mmole, and D-Ala^2-D-Leu-enkephalin (^{2}H-DADLE), ligand of OR of the δ-type in a concentration of 42 Ci/mmole (both were obtained from "Amersham," U.K.).

The serum corticosterone and cortisol levels were determined as described previously [1, 6].

EXPERIMENTAL RESULTS

The use of the radioreceptor method showed that displacing activity of OR ligands of the μ-type in the blood plasma of intact CBA and C57BL/6 mice was for practical purposes the same (99 ± 5 and 95 ± 6 pmole-equivalents DAGO/ml respectively). Meanwhile plasma levels of activity of OR ligands of the δ-type in CBA mice were almost twice as high as those for C57BL/6 mice (248 ± 7 and 145 ± 5 pmole-equivalents DADLE/ml respectively).

Changes in OR ligand activity in the blood after footshock stress followed a complex time course. During the first hour after the end of the procedure activity of OR ligands of the μ-type increased. After 1-3 h a decrease was observed in the values of this parameter, and finally, 10-24 h after the procedure activity of OR ligands of both μ- and δ-type in the plasma increased (Table 1).

The relatively low magnitude of the changes described and, in particular, the increase in activity of OR ligands of the μ-type of not more than 1.5-2 times was evidently the result of two factors. First, values of the parameters determined by the radioreceptor method reflect total activity of several plasma opioids, whose changes may have a different kinetics after the procedure. Second, maximal release of opioids in response to stress may perhaps take place in the earlier stages after the beginning of electrical stimulation.

Injection of dalargin led to weakening of the changes taking place in the endogenous opioid system under footshock stress conditions. This effect, recorded during assessment of activity of OR ligands of μ- and δ-types was observed in both early and late stages after the procedure on both lines of mice (Table 1).

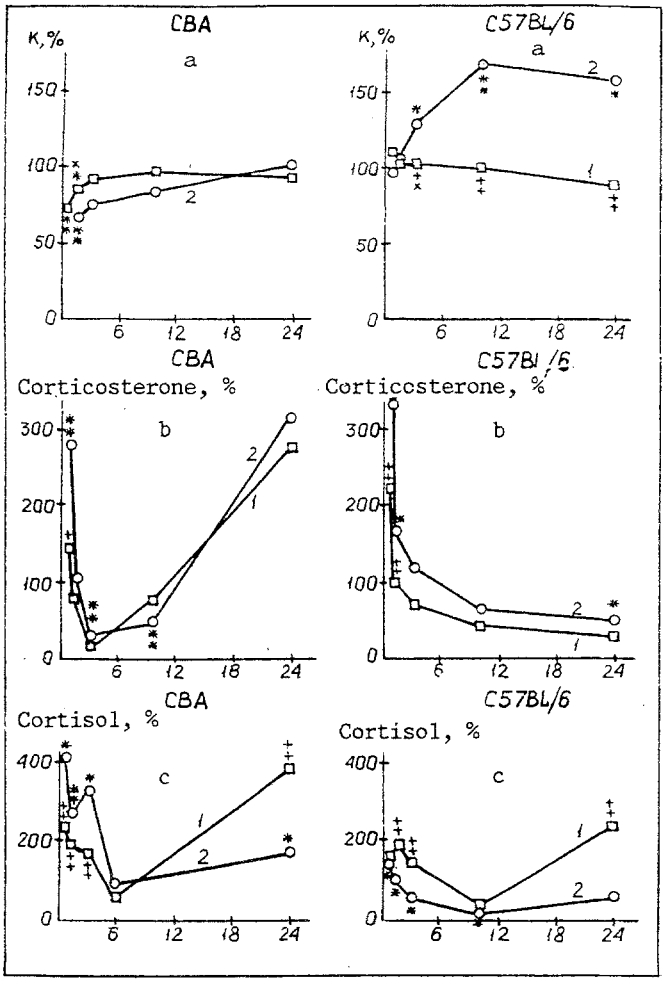

Fig. 1. Action of dalargin on ratio of values characterizing activity of OR ligands of μ- and δ-types (a); corticosterone (b) and cortisol (c) levels. Values of parameters recorded in intact mice at time of sacrifice of experimental animals taken as 100%. Value of K (δ/μ) was 2.5 ± 0.1 and 1.5 ± 0.1 in CBA and C57BL/6 mice respectively. Values of serum corticosterone concentrations varied within the range 62.2-112.3 and 146.5-253.4 ng/ml in CBA and C57BL/6 mice respectively. Values of cortisol concentrations were 29.7-50.3 and 100.4-161.7 nM in CBA and C57BL/6 mice respectively. Abscissa, time after end of electrical stimulation (in h). 1) Footshock stress + dalargin, 2) Footshock stress + physiological saline. *$p < 0.05$ compared with values recorded in intact animals; **$p < 0.05$ compared with values recorded in animals receiving physiological saline after exposure to shock.

Values of the coefficient reflecting the ratio of activities of OR ligands of μ and δ-types in the blood plasma are evidence of the presence not only of quantitative, but also of qualitative changes in the state of the opioid system of the experimental animals exposed to stress. For instance, in the early stages after exposure of CBA mice the value of the coefficient fell. The opposite effect was observed as a result of exposure of C57BL/6 mice to shock: not only was the coefficient not reduced, but it actually was increased starting 3 h after the end of electrical stimulation. The normalizating action of dalargin on this parameter was observed in experiments on both lines of mice. During development of the stress reaction dalargin raised the lowered values and lowered the raised values of the coefficient (Fig. 1a).

At different times after exposure to shock an increase in the blood cortisol level also was observed, and it was more marked in CBA mice. In the animals of this line 10 h after the end of stimulation of the limbs normalization of the blood hormone level was observed, whereas in C57BL/6 mice it fell. The cortisol concentration in CBA mice rose again 24 h after exposure to shock, whereas that of the C57BL/6 mice returned to normal. Injection of dalargin caused opposite effects on the cortisol level in the early stages (1-3 h) after electrical stimulation in mice of the different lines. Meanwhile, 24 h after stimulation of the limbs injection of dalargin led to a significant increase in the cortisol concentration in animals of the two lines (Fig. 1c).

The experiments thus showed that changes taking place in the state of the opioid system during the development of footshock stress cannot be interpreted as "activation" or "exhaustion." Changes take place in the relations between activity of ligands of the principal types of OR. These changes are characterized by interlinear differences. Significant interlinear differences also are characteristic of the time course of development of the late effects of footshock stress on the blood corticosterone level of the animals.

Injection of dalargin reduced the magnitude of the quantitative and qualitative changes taking place in the state of the opioid system during all stages of development of footshock stress studied, and it partially prevented elevation of the corticosterone level observed in the early stages after exposure to shock. Despite general features observed in the action of dalargin on opioid and steroid metabolism in mice of the two lines studied, interlinear differences were observed in the effects of dalargin.

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