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The Effect of Some Botanical Preparations on the Production of Lymphocyte-Activating Factor by Mouse Macrophages after Rotation Stress

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It is found that a short-term rotation stress triggers the production of lymphocyte-activating factors by peritoneal macrophages of (CBA×C57Bl/6) F_1 mice and raises blood levels of interleukin-1α and corticosterone. Botanical preparations administered to unstressed animals induce no secretion of lymphocyte-activating factors by macrophages and do not change blood levels of interleukin-1α and corticosterone. The herbals limit the stress-induced production of lymphocyte-activating factors by peritoneal macrophages.

Key Woords *interleukin-1α; lymphocyte-activating factor; botanical preparations, stress*

Botanical preparations (BP), complex naturally occurring compounds, have recently found wide application as adaptogens producing a complex effect on the organism's resistance to various damaging factors [1,2,11]. However, the mechanisms of their influence on resistance to stress are unclear. From the scant literature the modulation of the production of cytokines [13], including interleukin-1 (IL-1) [8], can be assumed to be one of the mechanisms responsible for the effects of BP.

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MATERIALS AND METHODS

Male (CBA×C57Bl/6) F_1 mice weighing 18-20 g were maintained at room temperature and on 12-h light-darkness cycles with free access to food and water. All experiments were started at 10 a.m. Stress was induced by rotation at 78 rpm for 1 h: 10-min rotations with 5-min breaks. The animals were decapitated 0, 0.5, 1, 2, 24, and 48 h after stress, and blood was collected to determine the hormone and IL-1α concentrations. Intact mice were not exposed to rotation stress.

Aqueous decoctions (1:20) of the underground parts of *Eleutherococcus senticosus*, *Aralia mandschurica*, and *Rhaponticum carthamoides* were used. The

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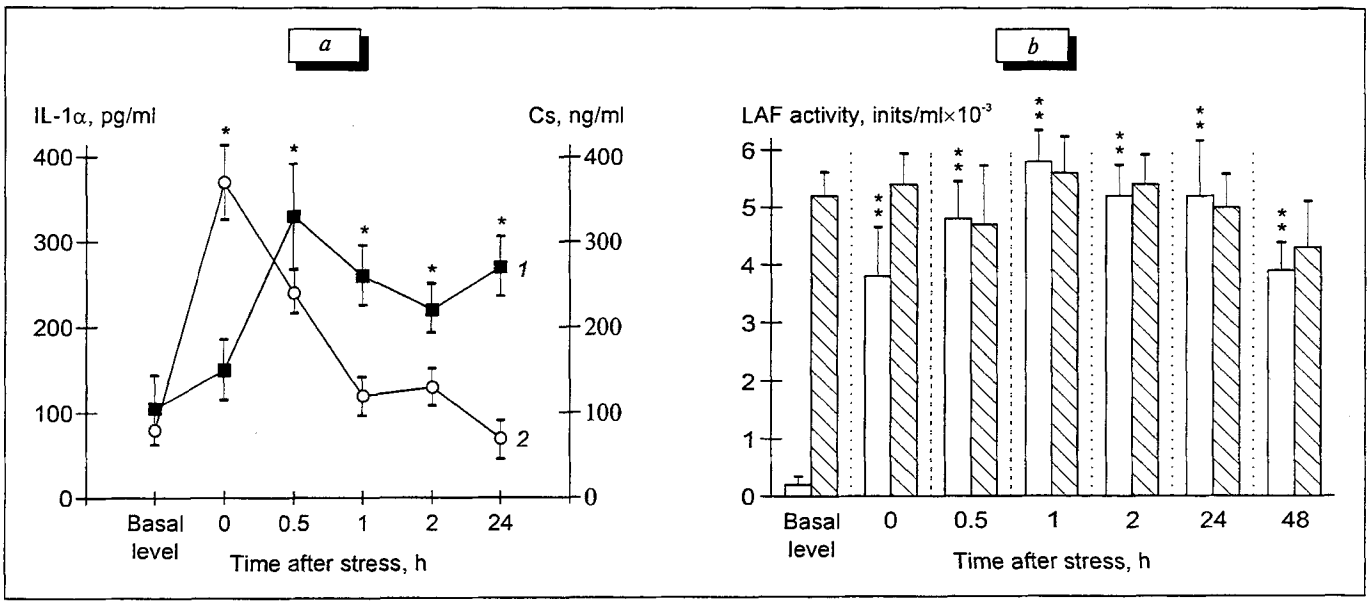

Fig. 1. Effect of rotation stress on plasma concentration of IL-1α (*1*) and serum concentration of corticosterone (Cs) (*2*) in mice (*a*) and on LAF production by peritoneal macrophages (*b*). White bars: without stimulation, shaded bars: stimulation with *St. aureus*. **p*<0.05; ***p*<0.01 compared with intact animals (basal level).

decoctions were prepared according to the Tenth State Pharmacopeia and stored in a cool, dark place for not more than 2 days. The preparations were administered via a semirigid gastral tube in a dose of 0.75 g dry matter/kg body weight in a volume of 0.3 ml per mouse every day during a 7-day period. Control animals were given the same volumes of boiled water via the tube. Peritoneal cells were collected immediately after decapitation. Adherent cells, 95% of which were peritoneal macrophages, were incubated without stimulation in a concentration of 3×10^6 cells/ml for 18 h at 37°C in medium 199 supplemented with 15% fetal calf serum (Sigma) and 100 U/ml penicillin in the presence of *Staphylococcus aureus* killed by heating (20 microorganisms per phagocyte). The cellular composition of the suspension was controlled by histochemical identification of nonspecific esterase [5].

The production of lymphocyte-activating factors (LAF) by macrophages was assessed by the comitogenic activity of the macrophage-conditioned medium towards the proliferation of murine thymocytes stimulated by suboptimal doses of lectins [10]. The plasma concentration of IL-1α was determined radioimmunologically using standard RIA kits (Amersham). The serum corticosterone level was determined radioimmunologically using standard kits produced by the Institute of Experimental Pathology and Therapy (Sukhumi). The differences between the experimental and control groups were analyzed using Student's *t* test.

RESULTS

Culture medium conditioned by resident peritoneal macrophages of intact mice displayed no LAF activity. After stimulation by staphylococci, these cells secreted LAF (Fig. 1, *a*). Immediately after rotation and during the subsequent 48-h period, murine macrophages produced LAF without additional stimulation. The maximum LAF activity was observed 0-1 h after stress. Staphylococcal stimulation of macrophages obtained

TABLE 1. Blood Concentrations of Corticosterone and IL-1α and LAF Activity of Culture Medium Conditioned by Mouse Peritoneal Macrophages after a 7-Day Administration of Botanical Preparations

Experimental group	Concentration		LAF activity, units/ml×10⁻³	
	corticosterone, ng/ml, in serum	IL-1α, pg/ml, in plasma	without stimulation	after stimulation by *St. aureus*
Intact (18)	76±15	82±11	0	3.8±0.4
H_2O (18)	88±21	84±24	0	4.3±0.5
Aralia (15)	98±18	104±28	0	6.2±0.8*
Eleutherococcus (15)	82±16	107±32	0	4.8±0.6
Rhaponticum (15)	102±25	121±33	0	6.8±0.7*

Note. The number of animals in a group is given in parentheses. **p*<0.05 compared with the control.

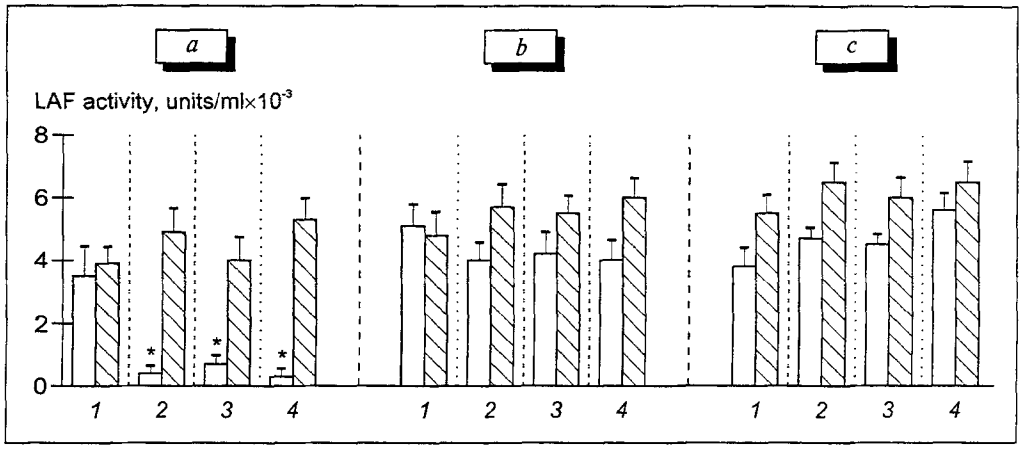

Fig. 2. Effect of botanical preparations on LAF production by murine peritoneal macrophages 48 h (*a*), 24 h (*b*), and 0 h (*c*) after rotation stress. White bars: without stimulation, shaded bars: stimulation with *St. aureus*. *1*) control (H_2O); *2*) *Aralia*; *3*) *Eleutherococcus*; *4*) *Rhaponticum*. *$p<0.05$ compared with the control.

from stressed mice induced no significant increase in LAF production compared with unstimulated cells (Fig. 1, *a*). Thus, a short-term rotation stress triggered the production of LAF in murine peritoneal macrophages.

Rotation stress induced a considerable increase in the plasma content of IL-1α, which reached the maximum 0.5 h after stress and remained significantly elevated during the following 48 hours (Fig. 1, *b*). The serum corticosterone concentration markedly increased 0 and 0.5 h after stress, indicating the development of a stress reaction, after which it returned to the baseline level within one hour (Fig. 1, *b*). Administration of BP to unstressed animals for 7 days did not induce LAF production by peritoneal macrophages; however, the decoctions prepared from *Aralia* and *Rhaponticum* significantly modulated the macrophagal response to staphylococci (Table 1). Blood contents of corticosterone and IL-1α in unstressed animals given BP did not change significantly compared with those in intact and control animals. The macrophages of mice given BP before rotation ceased LAF production without additional stimulation by the 48th hour after the stress, while the macrophages of mice given water continued to produce LAF (Fig. 2). Nevertheless, after administration of both BP and water before stress, the dynamics of LAF production by peritoneal macrophages stimulated by staphylococci remained unchanged (Fig. 2), indicating that their functional resources were preserved under these conditions.

From these findings it can be concluded that the rotation stress modeled in the present study induces macrophagal secretion of LAF, the most important of which is IL-1α. Increased production of IL-1α was confirmed by direct measurements of its concentration in the plasma.

It is known that various types of stress stimulate or inhibit the production of IL-1α by various cell systems [3], which may be due to a varied intensity and duration of the rise in the levels of glucocorticoid hormones. In our model, synthesis of IL-1α and LAF was induced against the background of a pronounced but brief increase in the blood corticosterone concentration, at which it stimulated proliferation and optimized the humoral immune response [4,9,12].

Although LAF, including IL-1α, are important modulators of defense reactions of the organism, their effects on these reactions are ambivalent: excessive or long-term production of LAF affects a number of the organism's systems and functions [6,7]. Therefore, the fact that BP shorten the period of stress-induced LAF production is apparently a manifestation of their stress-protective activity. It should be noted that the BP studied did not alter blood levels of IL-1α and corticosterone in unstressed mice, which indicates that they do not interfere with normal physiological processes; however, they did modulate LAF secretion induced by stress or staphylococci.

Our results confirm the hypothesis that the modulating influence of BP on the production of LAF, including IL-1α, may be one of the mechanisms underlying their effect on resistance to stress.

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Comparison of Lipid Peroxidation Parameters in the Heart, Liver, and Brain of Rats with Different Degrees of Resistance to Hypoxia

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The relationship between the intensity of lipid peroxidation and the activity of the antioxidant system in the heart, liver, and brain is studied in male Wistar rats with low and high resistance to hypoxia tested by being "raised" to an altitude of 11.5 km and in intact outbred rats. It is found that in all groups of rats the content of lipid peroxidation products is highest in the liver, lower in the heart, and lowest in the brain. In all groups, the rate of the ascorbate-induced lipid peroxidation is highest in the brain, lower in the liver, and lowest in the heart. The activity of the antioxidant system is highest in the brain, lower in the liver, and lowest in the heart of low-resistance and outbred rats, while in high-resistance rats it is the same in all the organs. Thus, the difference in the parameters of lipid peroxidation and, particularly, of the antioxidant system in the studied organs is most pronounced in rats with a low resistance to hypoxia.

Key Words: *lipid peroxidation; antioxidant system; hypoxia; low resistance; high resistance*

The brain is known to be the organ most sensitive to hypoxia, followed by the heart [8] and then the liver. In the heart, irreversible damage develops after 20-60 min of ischemia [15,16] and in the liver after 2-3 h [14]. The sensitivity of brain mitochondria to oxygen deficiency is higher than that of liver mitochondria [6]. However, in terms of the time of the initial significant increase in the content of lipid peroxidation (LPO) products in ischemia, these organs can be arranged as follows: brain (5 min)>liver (15 min)>heart (30 min) [2]. The different levels of sensitivity of these organs to ischemia may be associated with the initially different intensity of LPO and the activity of the antioxidant system (AOS) in them. In fact, the content of primary and secondary LPO products is highest in the liver, lower in the heart, and lowest in the brain; the antioxidant activity in the heart is considerably higher than that in the liver [2]. It can be assumed that the relationship between LPO intensity and AOS activity in the organs

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