CHARACTERISTICS OF SOME PARAMETERS OF THE IMMUNE STATUS, AND OPIOID AND INTERFERON SYSTEMS OF ATHLETES

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The immunomodulating effects of stress have been described in a number of publications. Meanwhile the neurohumoral mechanisms of development of these effects have not been adequately studied [3, 6]. The important role of opioid peptides in regulation of stress reactions [4, 5] and of immunomodulation [2, 8], established in recent years, has proved the basis for the suggestion that the opioid system is involved in the development of stress-induced disturbances of immunity [2]. In the investigation described below a parallel study was made of several parameters characterizing the immune status and also the function of the opioid and interferon systems of athletes. Choice of this test object was determined by evidence of the participation of opioids in the response of the body to psychophysical stress [4, 5, 7], and also results indicating the development of disturbances of immunologic reactivity of individuals under conditions of inappropriate physical exertion [6].

EXPERIMENTAL METHOD

Two groups of skaters (nine men and 15 women aged 18-24 years, engaged in the sport for 7-13 years) and healthy men and women of the same age, not engaged in sport (control group – 16 persons) were studied. The athletes were tested after a rest day. For 2 weeks before the investigation the skaters were trained at lowered intensity. The investigation of the skaters was preceded by a 2-week cycle of arduous training exercises (initial data). The state of the male athletes also was tested 10 h after training including physical exertion close to the upper limit of endurance, and also 8 h after intensive psychoemotional stress due to responsible competitions.

Blood was taken from a vein. The anticoagulant was 0.15 M EDTA (1:10). Samples intended for determination of opioid activity of plasma ligands contained the protease inhibitor bacitracin ("Sigma," USA) in a final concentration of 200 μg/ml. Plasma, obtained by centrifugation of blood (1000 g, 10 min, 2°C) was frozen and kept at −20°C before the extract was obtained. During extraction the samples were placed in boiling 0.25M acetic acid (1:10) for 10 min, cooled, and centrifuged (20,000 gm, 30 min). The supernatant was lyophilized and used for receptor radioimmunoassay.

The coarse membrane fraction of rat brain cells was isolated and RIA of the displacing activity of opioid receptor ligands (ORL) was determined as described previously [9]. ^{3}H-D-Ala^2-D-Leu^5-enkephalin (^{3}H-DADLE; 42 Ci/mmole, "Amersham," England), interacting selectively with opioid receptors of δ-type, was used as the labeled ORL. The concentration of ^{3}H-DADLE was 4 nM. Specific binding of ^{3}H-DADLE was determined as the difference between binding in the absence and presence of 2 μM DADLE ("Serva," West Germany) in the reaction medium.

The percentage and absolute numbers of lymphocytes capable of forming active and late rosettes (E_a- and E-RFC) with sheep's red blood cells, in the blood, proliferative activity of lymphocytes stimulated by phytohemagglutinin (PHA; "Difco," 20 μg/10^6 cells), the serum

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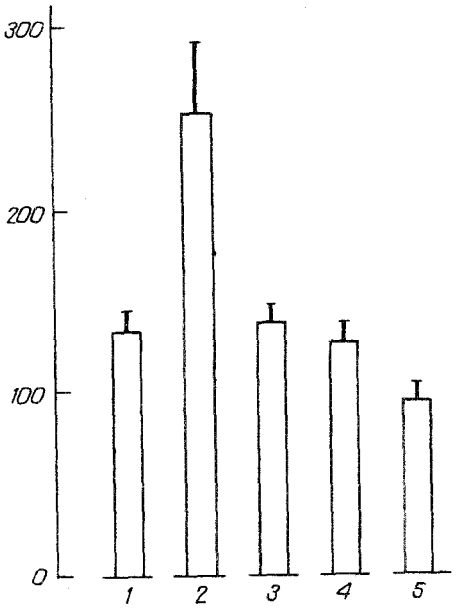

Fig. 1. Relative percentage of E_a-RFC (ordinate) in blood of healthy donors tested. 1) Control group; 20 skaters, stage of relative rest; 3) skaters, stage of relative rest; 4) 10 h after intensive training; 5) 8 h after important competition. Mean values ± standard error of the mean are given. *p < 0.05, **p < 0.01. Compared with value recorded in control group; +) p < 0.05, ++) p < 0.01 compared with value recorded in same athletes at stage of relative rest.

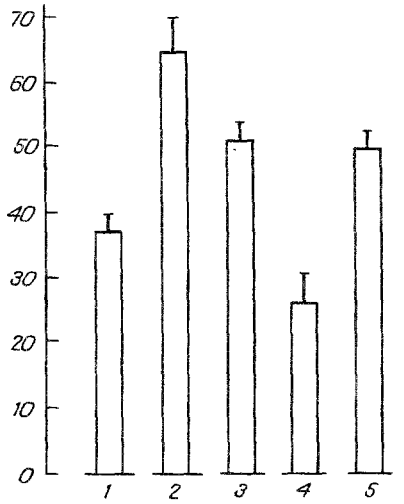

Fig. 2. Displacing activity of opioid receptor ligands of δ-type in blood plasma. Ordinate, pmole-equivalent DADLE/ml plasma. Remainder of legend as to Fig. 1.

interferon (IFN) concentration, and the ability of lymphocytes in vitro to produce α-IFN in response to viral stimulation and γ-IFN in response to stimulation by PHA, were estimated as described previously [1, 6].

EXPERIMENTAL RESULTS

Investigation of the athletes in the initial state revealed that parameters characterising proliferative activity and interferon production in the women were unchanged compared with the control. However, in men exposed to more arduous training exercises, the level of stimulated proliferative activity and of α-IFN production was significantly depressed

(28,898 cpm/10^6 cells and 27 U/ml in athletes compared with 110,918 cpm/10^6 cells and 64 U/ml in the control ($p < 0.01$). Synthesis of γ-IFN by the skaters' lymphocytes was virtually completely blocked. One possible cause of the development of the changes described above in the immune status of the male athletes may be connected with the use of unjustifiably arduous physical exercises in the training period.

During investigation of the skaters in the initial state an increase was observed in the relative percentage of E_a-RFC (Fig. 1) and in plasma ORL activity (Fig. 2). These data are in agreement with results indicating a raised level of opioid peptides in the blood of athletes [7] and also with the generally accepted view of the training influence of adequately graded physical exercises on the immune status. Meanwhile, in the group of skaters tested in the stage of more arduous training exercises, a less significant increase in the relative percentage of E_a-RFC was observed ($p < 0.05$; Fig. 1), but the displacing activity of the plasma did not differ from the control level (Fig. 2).

This investigation yielded results which can be regarded as essential evidence of the participation of the opioid system in regulation of the immune status. Positive correlation was found between the percentage content of E_a-RFC and the level of displacing activity of ORL in the athletes' blood plasma ($p < 0.01$). These data were obtained in the study of two independent groups of athletes, in a state of relative rest, and they are in good agreement with results indicating the ability of ORL of the δ-type and, in particular, of methione-enkephalin to stimulate the E_a-RFC level both *in vitro* and *in vivo* [2, 8].

In the study of the effect of arduous physical exercise on the athletes, 9 h after intensive training exercises, besides inhibition of the proliferative activity of lymphocytes stimulated by PHA, and of γ-IFN production, described in the initial state, $p < 0.05$), and so also was the ability of the lymphocytes to synthesize α-IFN (14.5 U/ml compared with 27.2 U/ml in the initial state, $p < 0.05$). The displacing activity of ORL in the plasma under these circumstances was virtually identical with its initial level (Fig. 2).

After competitions with the severe psychoemotional stress characteristic of them, the ability of the lymphocytes to effect active rosette formation corresponded with the initial level (Fig. 1). However, under these conditions α-IFN production was observed to be depressed down to its initial level, and activity of ORL in the plasma also was considerably reduced (Fig. 2), possibly as a result of exhaustion of the opioid system under conditions of stress.

It can be concluded from this investigation that outside the period of direct exposure to stress there is positive correlation between ORL to the δ-type, which nowadays are regarded as involved with stress-limiting effects [4, 5], and the level of active rosette formation, which is an integral characteristic of cellular immunity. It was also shown that different types of stress lead to different types of disturbances of immune homeostasis, possibly due to different mechanisms of their development.

In the whole the results suggest that during chronic exposure to frequently graded physical exercises the opioid system stimulates adaptive modifications of immunity. Meanwhile the action of excessively arduous and prolonged exercises leads to exhaustion of the opioid system, and this is one possible cause of the breakdown of adaptation and the development of secondary immunodefinciencies in athletes.

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