to hormones [7]. Elevation of the glucocorticoid level up to certain limits may lead to recirculation and the outflow of mature T cells to the periphery [2]. Our results are evidence that the thymus, acting through peptides in the composition of tactivin, can modulate both the functional activity of the adrenals and the response of these glads to ACTH; in turn, this is an additional and by no means unimportant factor in the regulation of the immune status.

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EFFECT OF HIGH- AND LOW-MOLECULAR-WEIGHT SOLUBLE BONE MARROW FACTORS ON ANTIBODY FORMATION AND PAIN SENSITIVITY IN ANIMALS

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Low-molecular-weight peptides of bone marrow origin – myelopeptides (MP) – have a wide spectrum of immunoreulatory action. They are known to stimulate antibody formation [5], to affect the functional activity of T cells [1] and macrophages [2], and to influence cell differentiation [6]. Meanwhile MP can induce a naloxone-dependent hypoalgesic action [3, 4]. The writers showed recently that low doses of MP lead to the development of hyperalgesia in animals. These facts raise the fundamentally important question of interconnection between antibody-stimulating activity of MP and their activiites affecting pain sensitivity.

The aim of this investigation was to compare the effects of three different fractions of supernatant of a bone marrow cell culture on antibody formation and pain sensitivity in mice.

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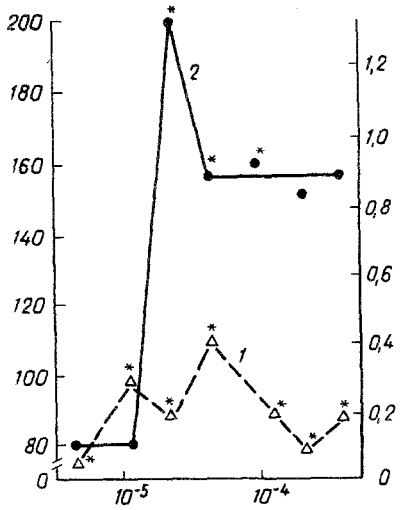

Fig. 1. Effect of fraction No. 1 on pain sensitivity (1) and antibody formation 92) in mice. Here and in Figs. 2 and 3: abscissa, dose of fraction No. 1 (in g/mouse); ordinate, on left – change in latent period of nociceptive response (in percent of control), on right – coefficient of stimulation of antibody formation, calculated as ratio of number of AFC in experiment to number of AFC in control. *p < 0.05.

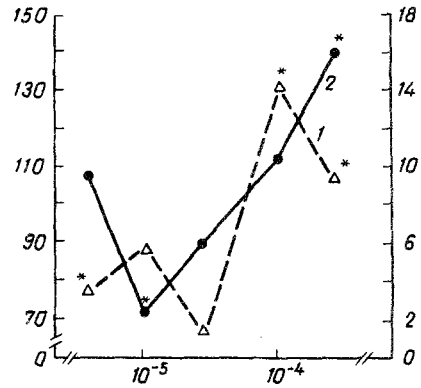

Fig. 2. Effect of fraction No. 3 on pain sensitivity (1) and antibody formation (2) in mice. Legend as Fig. 1.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL)F_1 hybrid mice weighing 18-22 g. Bone marrow mediators were injected once, intraperitoneally in different doses, in 0.2 ml of physiological saline. Control animals received 0.2 ml of the solvent. At various time intervals (from 15 min to 4 h) after injection of the test substances, the thresholds of pain sensitivity of the mice was determined by the hot plate method [7]. Each animal was tested once. The same mice, 24 h after injection of the test substances and determination of the pain threshold, were immunized intraperitioneally with a suboptimal dose ($5 \cdot 10^6$) of sheep's red blood cells (SRBC). The number of antibody-forming cells (AFC) was determined 5 days later in a suspension of spleen cells [8]. Individual fractions of bone-marrow mediators were isolated from the supernatant of cultures of hot bone marrow cells, concentrated tenfold. The bone marrow cells were cultured in a concentration of 10^7/ml for 20-22 h in serum-free medium RPMI-1640 in the presence of L-glutamine (2 mM), 10 mM HEPES, and 100 U/ml of penicillin. Three fractions were isolated by gel-filtration on Sephadex G-25 (fine), equilibrated with physiological saline (pH 7.2), from the supernatant: No. 1) with mol. wt. of 40-150 kD, No. 2) with mol. wt. of about 2 kD, and No. 3) with mol. wt. of under 1.0 kD). Fractions Nos. 2 and 3 of the bone marrow mediators are known as

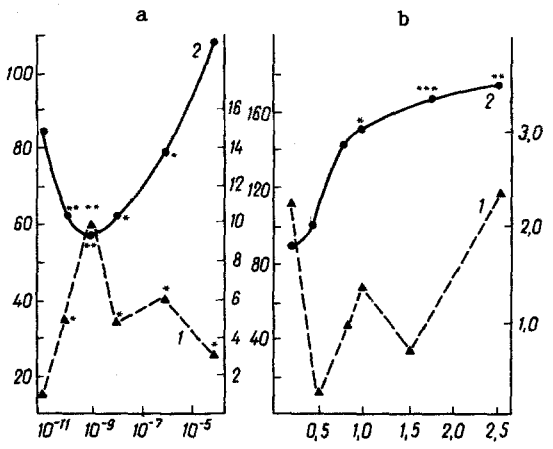

Fig. 3. Effect of fraction No. 2 on pain sensitivity (1) 30 min (a) and 2 h (b) after injection, and on antibody formation (2) in mice.

myelopeptides. The fractions thus obtained were lyophilizied, their protein content was determined by Lowry's method, and they were used in the experiments. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The time during which they remained functionally active was determined beforehand for all the test fractions.

Changes in the threshold of pain sensitivity under the influence of fraction No. 1 were observed 30 and 60 min after its injection in a does of $4.6 \cdot 10^{-5}$ g/mouse. The effect of fraction No. 1 was accordingly recorded 30 min after injection.

It will be clear from Fig. 1 that fraction No. 1, in doses of between $2.3 \cdot 1^{-5}$ and $11.5 \cdot 10^{-5}$ g/mouse caused an increase in the latent period of the nociceptive response by 30-95% compared with the control level. Higher or lower doses of fraction No. 1 had no statistically significant effect on the threshold of pain sensitivity.

Determination of the number of anitbody-forming cells in these animals on the 5th day of development of the primary immune response to SRBC showed that fraction No. 1 has a marked immunodepressive action. This fraction, between doses of $4.6 \cdot 10^{-6}$ and $4.6 \cdot 10^{-4}$ g/mouse depressed anitbody formation by 60-90% (Fig. 1).

Determination of the time when the effect of low-molecular-weight fraction No. 3 appeared showed that a change in the threshold of pain sensitivity was not observed until 30 min after its injection in a does of $3.4 \cdot 10^{-4}$ g/mouse. Unlike fraction No. 1, fraction No. 3 caused no change in pain sensitivity either 15 or 60 min after its injection.

Of the whole range of doses of fraction No. 3 tested (from $1.7 \cdot 10^{-5}$ to $3.4 \cdot 10^{-4}$ g/mouse) only two of them caused significant changes in the latent period of the nociceptive response (Fig. 2): fraction No. 3 in a dose of $3.4 \cdot 10^{-4}$ g/mouse lengthened the latent period by 40%, whereas in a dose of $3.4 \cdot 10^{-5}$ g/mouse it reduced it by 30%.

Doses of fraction No. 3 lowering pain sensitivity ($1.7 \cdot 10^{-4}$-$3.4 \cdot 10^{-4}$ g/mouse) increased antibody formation by 10-15 times (Fig. 2).

The change in pain sensitivity after a single injection of fraction No. 2 depended on dose. In a dose of 10^{-5} g/mouse, for instance, the latent period of the nociceptive response was reduced by 40% (Fig. 3a). The response began 15 min after injection and continued for not less than 2 h. The development of hypoalgesia after injection of milligram doses of fraction No. 2 did not begin until 2 h after the injection; the latent periods were increased in this case by 5-70%. It will be clear from Fig. 3 that close correlation exists between the change pain sensitivity and the effect of fraction No. 2 on the immune response. With lowering of the threshold of pain sensitivity by fraction No. 2 there was

an increase of 3-9 times in antibody formation. The exception was a dose of 10^{-4} g/mouse, after injection of which, stimulation of the immune response was not accompanied by any change in pain sensitivity. High doses of fraction No. 2, inducing hypoalgesia after systemic injection, had no significant effect on the primary immune response.

The results of these investigations showed that low-moelcular-weight fractions isolated from the supernatant of cultures of bone marrow cells (fractions Nos. 2 and 3) had a simultaneous regulatory effect on the primary immune response and on pain sensitivity. Each fraction tested had its own character of dependence on time and dose. However, strict correlation was found between the effect of the test fractions on pain sensitivity and their action on the immune response. Strengthening or weakening of the immune response corresponded to strengthening or weakening of pain sensitivity. For instance, fraction No. 2, containing a group of peptides with mol. wt. of about 2 kD (myelopeptides), in nanogram doses induced hyperalgesia. The same doses of fraction No. 2 had a marked antibody-stimulating action. Milligram doses of fraction No. 2 led to the development of hypoalgesia, but had no affect on antibody formation. Incidentally, in some experiments nanogram and microgram doses of fraction No,. 2 did not affect the threshold of pain sensitivity. In this case no change was observed in the development of the immune response.

An opposite action on pain sensitivity also was recorded after injection of fraction No. 3 with mol. wt. of under 1.0 kD. The hypoalgesic effect of fraction No. 3 was combined with immunostimulation. Meanwhile the development of hyperalgesia had no significant effect on the immune response. The hypoalgesic action of the high-molecular-weight fraction No. 1 was accompanied by an immunodepressive effect.

These data indicate close interconnection between such vitally important systems of the body as the pain monitoring and immune surveillance systems. However, the molecular structures responsible for this interaction remain unidentified: whether they are common to these two systems or whether each of them has its own concrete regulator.

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