EFFECT OF DALARGIN ON STRESS-INDUCED CHANGE IN MACROPHAGAL 5′-NUCLEOTIDASE ACTIVITY AND ENDOGENOUS CORTISOL LEVEL IN MOUSE BLOOD

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Much factual evidence has now been gathered on the important role of opioids in function of the neuroendocrine system [1, 4]. There is evidence that opioid peptides can influence stress-induced reactions [8, 10]. The immunomodulating activity of these compounds has been established [3] and the effect of opioid peptides on the mononuclear phagocyte system has been studied [13]. However, there have been only sporadic attempts to study relations between macrophages and the endocrine functions of the body under the influence of opioid peptides.

The aim of this investigation was to study the effect of dalargin, a synthetic Leu-enkephalin analog, on stress-induced changes in 5′-nucleotidase (5-NA) activity of peritoneal exudate macrophages (PEM) and on the endogenous blood cortisol level in mice of different lines.

EXPERIMENTAL METHOD

Male CBA and C57BL/6 mice (18-20 g) were kept in the animal house under standard conditions of light, temperature, and diet. The period of acclimatization of the animals before the beginning of the investigation was at least 2 weeks. PEM were obtained by the method in [2]. Ecto-5′-NA in the macrophages was determined by the method in [9].

Cortisol was determined by fluorescence immune analysis ("Delphia") based on competitive interaction between the cortisol molecule, immobilized on a solid phase, and europium-labeled cortisol [12].

To create of model of footshock stress, chambers made by the Diagnostika Research-Production Combine were used. The current was applied to the floor of the chamber under the following conditions: [(1.5 mA × 5 sec, interval of 5 sec) × 25 times, interval of 30 sec] × 3 times – total duration of the procedure 13 min.

Dalargin (5 μg/kg body weight) in 0.5 ml physiological saline in the experimental group or 0.5 ml 0.14 M NaCl in the control group, was injected into the venous sinus of the eye 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 exposure to stress.

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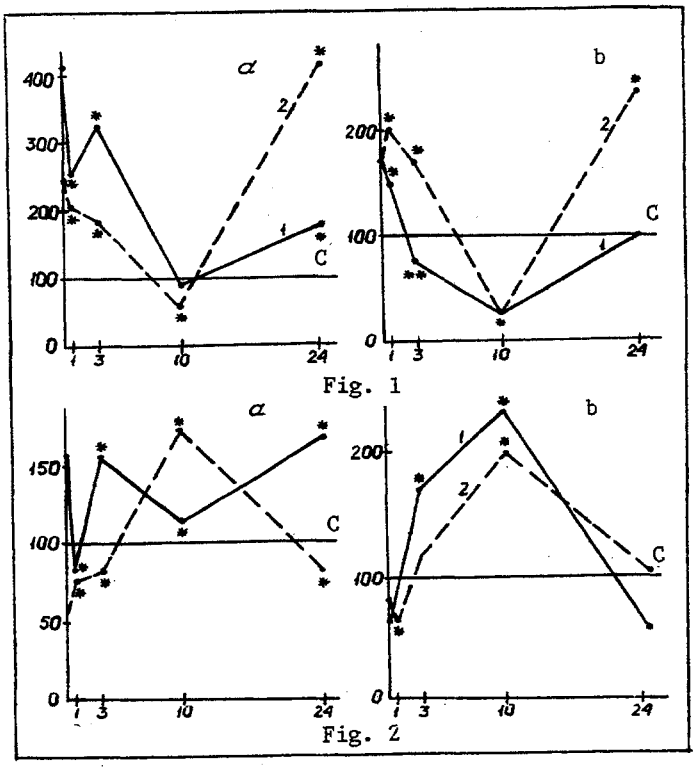

Fig. 1. Changes in blood cortisol level in CBA (a) and C57BL/6 (b) mice: 1) stress, 2) stress + dalargin, C) control. Abscissa, time (in h, after stimulation); ordinate, cortisol level (in % of C).

Fig. 2. Changes in 5′-NA activity of PEM in CBA (a) and C57BL/6 (b) mice. Ordinate, level of 5′-NA activity of PEM (in % of level of activity in control animals – C). Asterisk indicates values for which $p < 0.05$. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

The results of determination of the endogenous blood cortisol level in CBA and C57BL/6 mice after stress are given in Fig. 1. They show that 20 min after stimulation a significant increase in the blood cortisol level was observed in mice of both lines, and rather more marked in the CBA mice. After 1 h the cortisol level fell. In C57BL/6 mice, the blood cortisol after 3 h had fallen below the control level. At all subsequent times of investigation the cortisol level was lower than in the control animals. In CBA mice, on the other hand, after 3 h a very small and not significant increase in the hormone level was noted. By contrast with the C57BL/6 mice, in the mice of this line, the cortisol level did not fall below the control values at any time during the investigation. Toward 10 h after stress it had only moved close to the control level, and this was followed by a small increase toward the end of the 1st day.

In response to injection of dalargin into the stressed animals the cortisol level in mice of both lines showed no significant changes, and in its general features it repeated the dynamics of the cortisol level in the stressed animals. Not until 24 h after stimulation was a second increase in the blood cortisol level observed under the influence of dalargin in CBA and C57BL/6 mice.

The dynamics of 5′-NA activity of PEM in the stressed animals and under the combined influence of stress and dalargin is shown in Fig. 2. It will be clear from Fig. 2 that the curve of activity of this enzyme in the stressed CBA mice in its general features repeats the dynamics of the blood cortisol level. In C57BL/6 mice, on the other hand, it was apparently the mirror image of the change in the blood level of cortisol. Injection of dalargin into stressed C57BL/6 mice did not affect the character of the change in 5′-NA activity, while preserving the opposite relationship between changes in blood cortisol and the 5′-nucleotidase activity of PEM.

A different picture was observed in CBA mice. Injection of dalargin had a considerable effect on the character of the change in enzyme activity of PEM compared with the stressed animals. Moreover, dalargin changed the relations between the endogenous cortisol level and enzyme activity of PEM. For instance, whereas in the stressed mice positive correlation was observed between the above-mentioned parameters, under the influence of dalargin it became negative, just as in the CBA mice.

Correlation between enzyme activity of PEM and the blood cortisol level, revealed by this investigation, is evidently not accidental. This is shown by the definite synchronization of the two parameters in space and time. Furthermore, similar correlations were established in the case of immunomodulators of bacterial origin. Just as under stress, under the immunomodulating effect correlation was found between 5′-NA and the blood cortisol: positive in the CBA and negative in the C57BL/6 mice [7].

The existence of correlation of this kind between the level of 5′-NA activity in PEM, on the one hand, the blood cortisol level, on the other hand, and various immunologic parameters [5, 6], suggests that the adenosine system of the macrophages is one of the universal systems in the body participating in the realization of two-way connections between the immune and neuroendocrine systems. This state of affairs is embodied sufficiently completely in modern views regarding the structure of function of 5′-NA and its role in the regulation of the extracellular and intracellular adenosine level [15]. Adenosine as we know is one of the principal regulators of vital physiological processes, including immunologic. Adenosine also is involved in regulation of the functional activity of the neuroendocrine system [11].

It was thus shown for the first time that dalargin, a synthetic Leu-enkephalin analog, can modify the character of correlation between 5′-NA of PEM and the blood cortisol level in CBA mice, changing from positive into negative. This observation is evidence of a possible role of opioid peptides in the regulation of relations between phagocytic cells and, in particular, of the adenosine system of the macrophages, and the neuroendocrine system. The results thus obtained are in agreement with data in the literature on the close correlation existing between the adenosine and opioid systems [14].

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