Effect of Immobilization Stress on Macrophage 5'-Nucleotidase Activity and Endogenous Cortisol Level in CBA and C57Bl/6 Mice

G. B. Kirillicheva, N. P. Goncharov, V. V. Mit'kin, I. G. Baturina, G. V. Smirnova, M. V. Tomoshunas, and G. T. Sukhikh

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Key Words: *immobilization stress; 5'-nucleotidase; macrophages; cortisol*

The development of the general adaptation syndrome involves a restructuring of the intra- and intersystemic relationships. Every year brings an increase in the flood of information concerning the interaction between the phagocytosing cells and the neuroendocrine component of the regulation of immunity. However, the specific mechanisms and peculiarities of the interrelationship between these systems, especially in stress situations, still need further investigation.

This paper presents the results of a comprehensive study of the specific features of the effect of immobilization stress on 5'-nucleotidase (5'-N) activity in peritoneal exudate macrophages (PEM) and on the endogenous cortisol level in mice of different strains.

The development of the general adaptation syndrome involves an enhancement of the organism's resistance to conform to the qualitative and quantitative characteristics of the stimulus. The level of ecto-5'-N activity in the PEM represents one of the factors of the resistance to infection. There is now quite a large body of evidence of the extremely important role of the glucocorticoids in the development of the immunological mechanisms of resistance. The glucocorticoids occupy a unique position among the hormones controlling the system of mononuclear phagocytes. A study of the two mentioned indexes and their interrelationship is therefore of considerable importance for understanding the mechanisms of development of nonspecific resistance in the organism.

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences; Research Center of Mother and Child Health; Endocrinological Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Male mice of the CBA and C57Bl/6 strains were housed in a vivarium under standard conditions of light, temperature, and feeding. Before the start of the experiment the animals underwent acclimatization for at least two weeks. The stress stimulus was produced by immobilization in a special device. The duration of the immobilized state varied from 5 to 60 min. One to 20 min and one to 24 hours following the end of the stress stimulus the activity of 5'-N in the PEM [3] and the level of endogenous cortisol in the blood serum were measured. Cortisol was determined by fluorescent immunoassay ("Delphia"), based on the competitive interaction between solid-phase immobilized cortisol molecules and free europium-labeled molecules [4].

RESULTS

The results of PEM 5'-N activity and blood cortisol determination in CBA and C57Bl/6 mice are pre-

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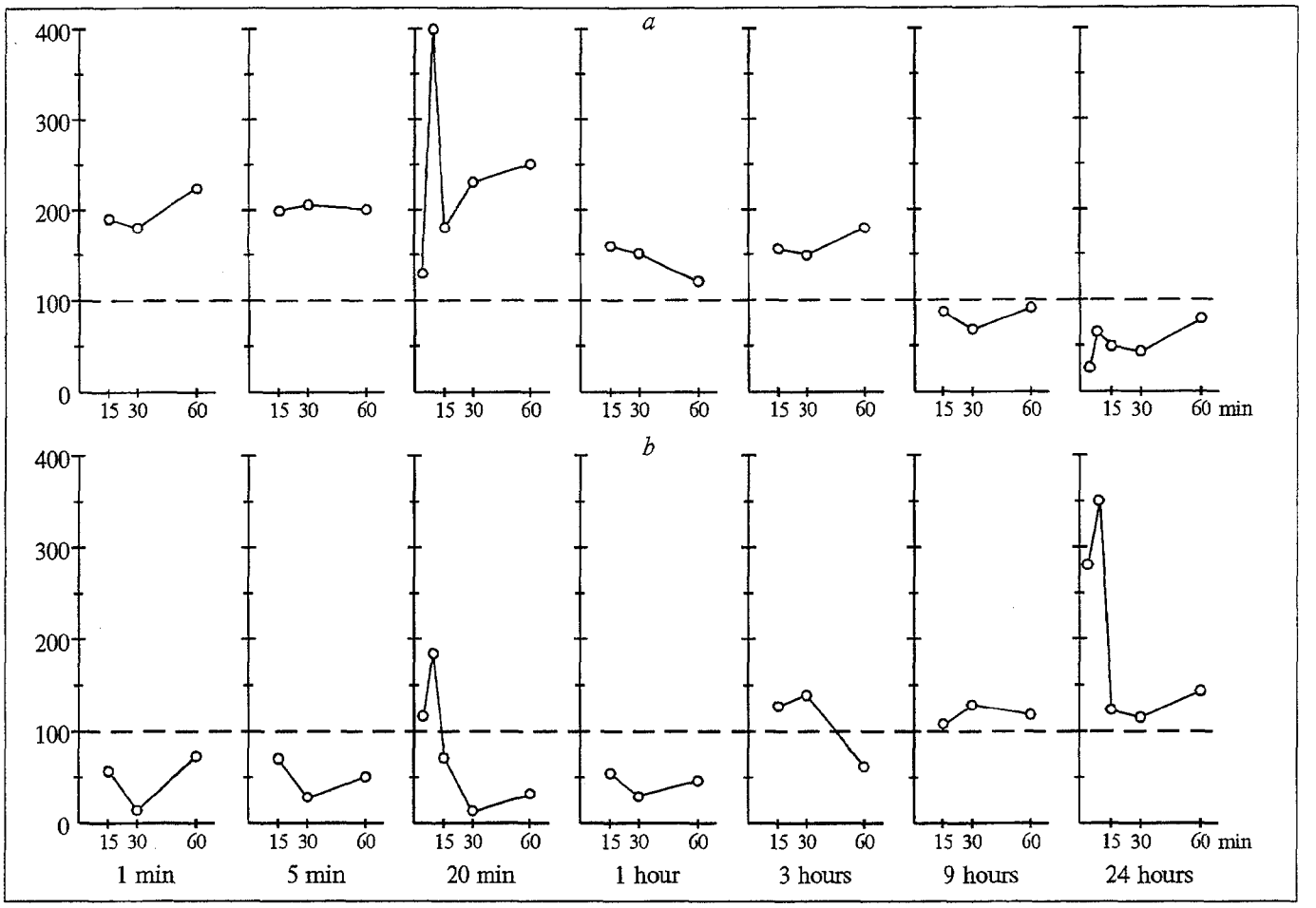

Fig. 1. Change of endogenous cortisol level (*a*) and PEM 5'–N activity (*b*) in CBA mice subjected to immobilization stress (percent of corresponding values in control animals).

sented in Figs. 1 and 2. It is seen that as early as in the first few minutes from the beginning of stress both indexes undergo significant changes. No direct correlation could be detected between the intensity of stress and the degree of change in the endocrine and macrophage parameters. The dose-effect curves for both were of a complex character, different in CBA and C57Bl/6 mice. In addition, the appearance of the curves changed depending on the period elapsing following the end of the stress stimulus, i.e., on the stage of stress. The dynamics of the processes shows the phase-dependent changes of the indexes. For instance, the rise of the cortisol level and drop in the 5'-N activity detected in the initial periods gave way to inverse changes in the later periods. It should be pointed out that in CBA mice the reversal of direction as compared to the control took place earlier (after approximately 9 hours) than in C57Bl/6 mice (after 24 hours). In other words, CBA mice exhibited a more mobile character of the parameters. Moreover, in the mice of this strain the degree of change in the macrophage and endocrine parameters was somewhat more pronounced than in C57Bl/6 mice.

Additional differences between the strains were revealed in the comparative analysis of the dynamics of 5'-N activity and cortisol level. Figure 1 shows that in the initial periods of the observation the change of 5'-N activity in the PEM of CBA mice is of a more monotonous nature, less prone to fluctuations, than the cortisol level. For instance, the profile of the dose-effect curve for 5'-N undergoes almost no changes in the period up to 60 min and assumes its mirror image only 3 and 9 hours following the stress treatment. The pattern of the dose-effect curve for cortisol differs for each successive period of observation.

On the other hand, in C57Bl/6 mice the pattern of the dose-effect curve is more labile and dynamic for the macrophage than for the endocrine parameter. Figure 2 shows that the direction of the dose-effect curve for 5'-N changes in each successive period of observation; however, the analogous curve for cortisol is of a constantly convex form during the period up to 60 min, becoming its mirror image only after 9 hours.

Thus, one of the manifestations of interstrain differences is an unequal dynamics of the hormone and macrophage values. For instance, CBA mice ex-

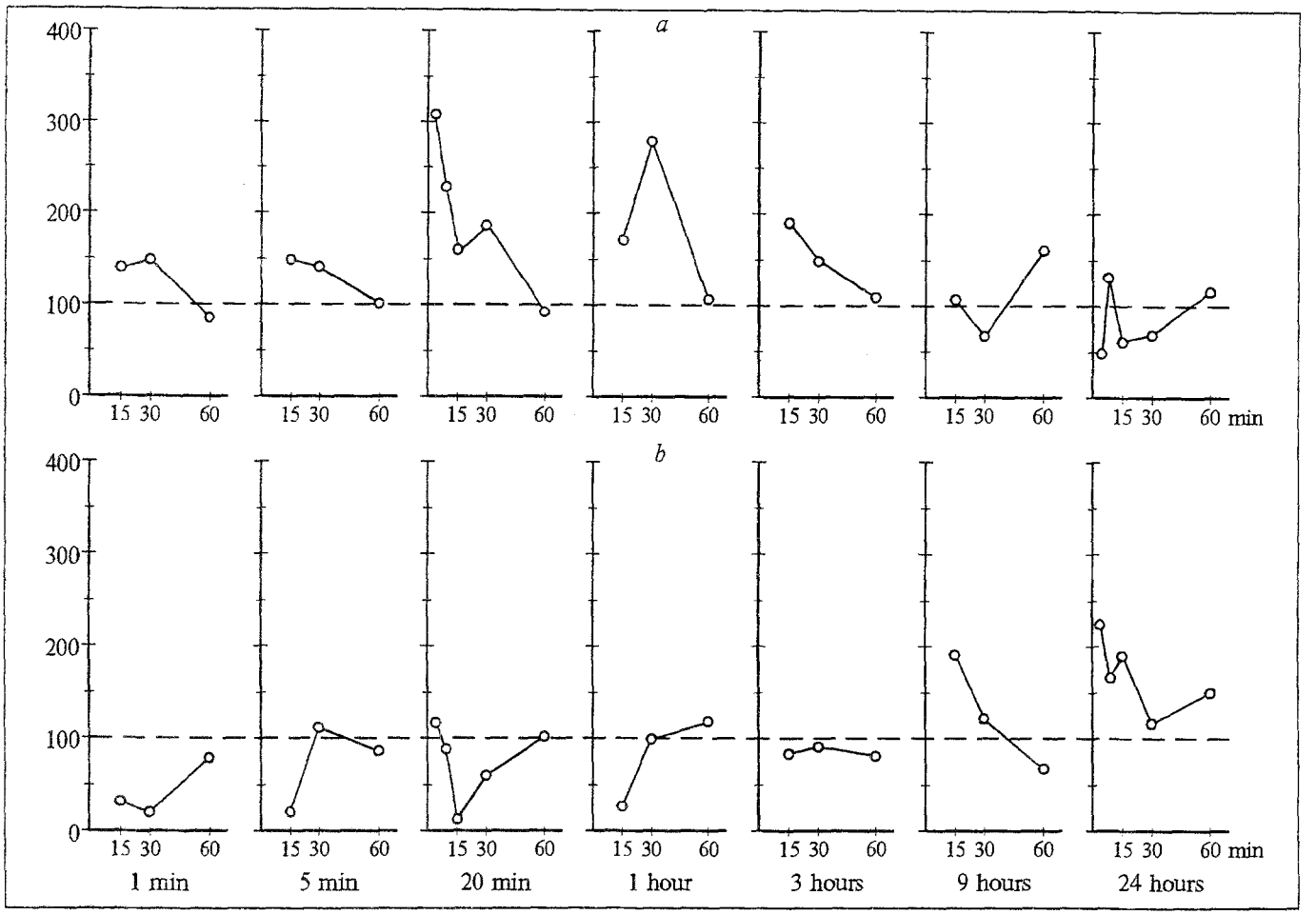

Fig. 2. Change of endogenous cortisol level (*a*) and PEM 5'–N activity (*b*) in C57Bl/6 mice subjected to immobilization stress (percent of corresponding values in control animals).

hibit a more monotonous pattern of changes in the PEM 5'-N activity, whereas in C57Bl/6 mice monotonous changes were observed in the cortisol level.

The analysis of the complex curves suggests that each value at any given time point is influenced by many factors, whose integral effect depends on both the intensity of stress and the genotype of the organism.

The results of this study are consistent with the current notion concerning the stagewise character of stress, and they are in agreement with the known trends of changes in the endocrine parameters. The data obtained by us for the first time on the effect of immobilization stress on 5'-N activity provide evidence that the changes in this metabolic index are governed by the same regularities.

Earlier it was shown that a stress stimulus [2] and challenge by bacterial immunomodulators [1] are accompanied by the appearance of a correlation between PEM 5'-N activity and endogenous cortisol level. This dependence was shown to be direct in CBA mice but inverse in C57Bl/6 mice.

The analysis of the results presented here shows that the correlation between PEM 5'-N activity and cortisol is not absolute but appears only at a certain stage of stress. It may be assumed that these indexes are under the common control of one or more particular regulatory factors. Depending on the specificity and intensity of the stress stimulus and the genotype peculiarities, these factors act in different combinations, thereby determining the patterns of the dose-effect curve and the correlative relationship between the two parameters.

Analysis of the data from the literature and our own results allows us to formulate a hypothesis about the leading role of the hypothalamus in the establishment of the correlative dependence between PEM 5'-N and blood cortisol.

This hypothesis does not contradict the earlier detected property of dalargine to change the direction of the relationship between the mentioned indexes [2]. The effect of opioid peptides on neuroendocrine regulation is known to be realized at the level of the hypothalamus.

Thus, this study has demonstrated the genotypic peculiarities of the macrophage and endocrine parameters in the response to a stress stimulus.

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Effect of Metalloorganic Immunomodulators on Susceptibility to Plague Toxin

G. B. Kirillicheva, I. G. Baturina, K. K. Rozhkov, and M. A. Ignatenko

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The use of immunomodulators (IM) is being increasingly extended to the treatment of various pathological states. Reports have appeared about their capacity to influence the susceptibility of the organism to various toxic stimuli.

The goal of this work was a study of the influence of IM of metalloorganic nature on the susceptibility of mice of different strains to "mouse toxin" (MT) of plague microbes. MT is a plague microbe-derived lethal protein toxin of high activity. Keeping in mind the diurnal variations in the organism's resistance to the toxic effect of various compounds, the experiments were carried out at different times of the day.

MATERIALS AND METHODS

Male mice of the CBA and C57Bl/6 strains and F_1 (CBA×C57Bl/6) were used. The animals were kept in a vivarium under standard conditions of light, heat, and feeding. The acclimatization period before the start of the experiments was not less than 2 weeks. The IM MOP-35 and MOP-79 were administered intravenously at 11:00 and 23:00 h. Control animals received saline.

MT was isolated and purified from the lysate of *Y. pestis* EK 76 cells [7]. The toxin was introduced intraperitoneally in doses of 0.5 and 1 mg per mouse, 5 min after IM infusion. Mortality in the experimental and control groups was recorded. Changes in the susceptibility to the toxic stimulus were estimated as the ratio of mortality in the experimental group to mortality in the control group and expressed in percent.

RESULTS

The results of the experiments examining the effect of MOP-35 and MOP-79 on the susceptibility of mice to MT are presented in Fig. 1 and Fig. 2.

As can be seen in the figures, as early as 5 min after IM intravenous infusion a reliable change of susceptibility to the toxic effect can be seen, depending on the time of day. The effect of IM on the susceptibility

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

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