The same tendency was traced in the response kinetics, except that the immunogenicity of $DA_{5.8}$-BSA after hyperimmunization (4 injections) attained that of $DA_{8.8}$-BSA and DA_{14}-BSA.

Thus, the immunogenicity of dopamine-protein conjugates increased in the following order: DA_4-BSA - $DA_{5.8}$-BSA - DA_{14}-BSA and decreased with a further increase of hapten valence. The optimal immune response developed at a hapten valence of 5.8-14. It may be supposed that the partial tolerogenic properties of thymus-dependent antigens such as dopamine-protein conjugates are accounted for both by specific processes (that are more typical for low-substituted antigens) and by nonspecific processes that are connected with alterations in the physicomolecular characteristics of antigen. The latter is directly connected with the mode and conditions of hapten-protein coupling. High-substituted conjugates (DA_{18-22}-BSA) acquire tolerogenic properties due to nonspecific mechanisms rather than to specific blocking of receptors.

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Effect of Defensins on the Blood Level of Corticosterone and the Immune Response During Stress

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An important role of the interaction between the neuroendocrine and immune systems in the formation of the defense response of the organism during stress is now commonly acknowledged [5,9]. Nevertheless, the specific mechanisms of this process remain the object of recent studies. In this connection, the data reported by Canadian scientists [15] on the ability of defensins (cationic cyclic polypeptides possessing antimicrobial properties and located in the granular apparatus of neutrophils and some macrophages [4,10]) to suppress adrenocorticotropic hormone (ACTH)-induced production of steroids by adrenal cells in culture are of certain interest. This property of the polypeptides has been termed by the authors corticostatic. The ability of defensins to exert a corticostatic effect has been regarded by researchers as one of the probable molecular mechanisms of the immune effect on the activity of the hypothalamus-pituitary-adrenocortical system (HPACS), which is based on the principle of negative feedback [8]. However, it is not yet certain whether such an adrenal-altering effect of defensins is true for the whole organism. We were the first to study the effect of exogenous defensins on some indexes of the organism's

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endocrine and immune state in experimental animals under conditions of stress- and ACTH-induced activation of HPACS [7,11]. Since under such influences the adrenal production of glucocorticoid hormones is intensified [5,11], such models may be considered proper for studying the corticostatic effect of defensins *in vivo*.

In the present work we studied the effect of defensins and determined their influence on the realization of the humoral immune response under conditions of immunosuppressive stress.

MATERIALS AND METHODS

Experiments were carried out on male (CBA3 C57Bl/6)F_1 hybrid mice weighing 18-20 g and Wistar rats weighing 180-200 g. The following models of stress were used in the study. In the mouse experiments a model of rotation stress was used. The duration of stress was 10 min at 78 rpm. Rabbit defensin in doses of 20 ng/g and 2 mg/g was administered intraperitoneally 10 min before stress. The blood for corticosterone determination was taken by decapitating animals 30, 60, and 120 min after stress. In the study of the effect of defensins on the immune response in mice, the model of combined stress was used. The animals, restrained in special stands, were placed in a refrigerator (+4-5°C) for one hour, and then the mice were moved in the same stands to premises at room temperature (18-20°C) for 24 h. During this day the animals received no food or water. Rabbit defensin in doses of 2 mg/g and 20 mg/g animal weight was administered intraperitoneally 10 min before combined stress. Sheep erythrocytes in a dose of 5×10^7 cells were administered directly after the end of the stress influence (1 day after defensin injection). On day 6 after immunization the animals were decapitated and blood was taken for antibody titer determination by the method of direct hemagglutination, as well as a spleen specimen for antibody-producing cells (APC) determination [1]. The rats were subjected to stress by 10-min cooling in a refrigerator (-20°C). Ten minutes before stress the animals were injected with rabbit defensin in a dose of 20 ng/g. After 30 min, the animals were decapitated, and the blood was taken for corticosterone determination. One of the stages of the work was to study the effect of defensins on the ACTH-induced increase of the blood level of glucocorticoid hormones. For this purpose, ACTH (Organon, Holland) in a dose of 0.02 IU/g was injected 30 min after defensin injection. Animals which received equal volumes of physiological saline instead of polypeptides served as controls. The serum level of corticosterone was radioimmunologically assayed by the method of competitive binding to the protein [2] with the aid of standard kits (obtained from the Sukhumi Research Institute of Experimental Pathology and Therapy). Defensins were obtained from the neutrophils of a 4-h aseptic peritoneal focus of inflammation induced by intraperitoneal injection of 300 ml sterile physiological saline with 5% starch in the rabbits (or 30 ml in mice) according to the following scheme. Cell suspension was disrupted by homogenization in 10% acetic acid (10 ml solution for 1 g cells). The homogenate was centrifuged at 25,000 rpm during 1 h. The supernatant was decanted and dialyzed against 5 volumes of 10% acetic acid in 250-9I dialysis tubes (Sigma) during 24 h. The dialyzed solution containing acid-soluble proteins and polypeptides of molecular weight less than 15,000 D was freeze-dried and applied onto a column packed with Akrilex P-10 (Reanal, Hungary) equilibrated with 5% acetic acid. The fraction of polypeptides with molecular weight of about 5000 D was freeze-dried and additionally purified on CM-cellulose (CM-52, Whatman, England) buffered with 0.02 M Na-acetate in 0.1 M NaCl (pH 4.5). Material from this fraction retained by the ion-exchanger was eluted from the column with 1 M NaCl. The material obtained was subjected to electrophoretic assay after Panyim [13] for assessing its purity. According to the results of the assay, the defensin preparations obtained were totally polypeptide fractions and consisted of 5components

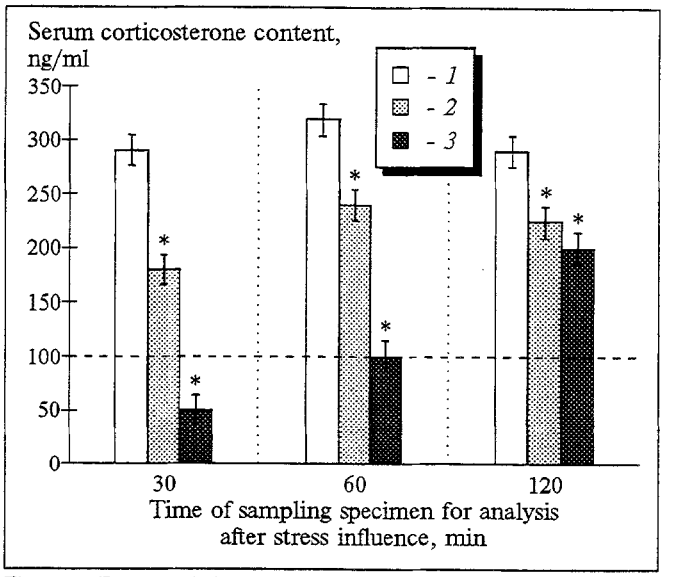

Fig. 1. Effect of defensins on changes of blood level of corticosterone in mice subjected to rotation stress. *1*: control, animals injected with physiological saline 10 min before stress; *2*: animals which received 20 ng/g rabbit defensin 10 min before stress; *3*: animals which recieved 2 μg/g rabbit defensin 10 min before stress. Asterisk: $p<0.05$ vs. control.

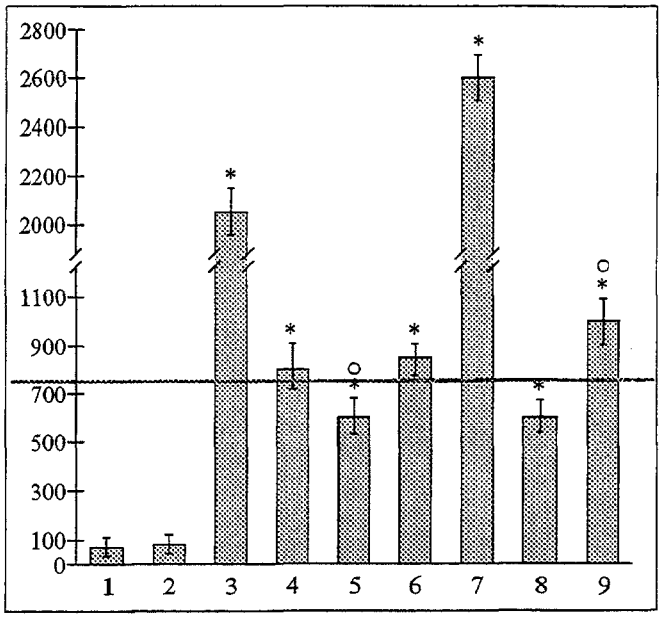

Fig. 2. Effect of defensins on ACTH-induced rise of corticosterone level in rats. Ordinate: serum content of corticosterone, ng/ml. Abscissa: animal groups. 1) intact animals; 2) animals injected with physiological saline; 3) animals injected intraperitoneally with 3 IU/rat ACTH; 4) animals which received 10 μg/g rabbit defensins; 5) animals which received 10 μg/g rabbit defensins 30 min before ACTH injection; 6) animals which received rabbit defensins in a dose of 5 ng/g; 7) animals which received rabbit defensins in a dose of 5 ng/g 30 min before ACTH injection; 8) animals which received rat defensins in a dose of 5 ng/g; 9) animals which received rat defensins in a dose of 5 ng/g 30 min before ACTH injection. Asterisk: $p<0.05$ vs. group 2; circle: $p<0.05$ vs. group 3.

in rabbit and of 3 in rats. In the present paper they are designated as defensin preparations.

RESULTS

Rabbit defensins administered before the rotation stress markedly decrease the stress-induced augmentation of glucocorticoid hormones in mice in comparison with the control group of animals (Fig. 1). Thirty minutes after the end of stress, the inhibiting effect of the polypeptides was particularly pronounced, a dose of 2 μg/g inducing a more marked response.

Similar changes of the blood level of corticosterone were detected in rats during the period prior to the influence of cold for the injection of defensins into the animals. The hormone content in such animals was almost 2 times lower than in the group just subjected to stress (62-72 ng/ml vs. 128-145 ng/ml). The qualitative stereotypy of hormonal shifts in the animals examined may provide evidence of uniformity of the morphochemical and physiological mechanisms involved in the formation of adaptive response to stress. Along with the Canadian scientists, we may assume that one of the targets of the effect of defensins introduced in the organism may be the ACTH receptors of the adrenal cortex, the synthesis and secretion of corticosterone [15] being inhibited by competetive binding of the polypeptides to them.

To elucidate the possible effect of defensin on glucocorticoid production at the adrenal level, the responses of glucocorticoids to polypeptides were studied in rats for the injection of ACTH (Fig. 2). It was shown that injecting the preparations of heterologous (group 4, 6) and homologous (group 8) defensins *per se* causes an increase of the blood concentration of corticosterone. This hormonal shift may be regarded as a particular manifestation of the neuroendocrine response of the organism to stress stimuli, including those of polypeptide origin [6]. Rabbit defensin in a dose of 10 μg/g markedly reduces the ACTH-induced response of the organism (group 5) but fails to produce any inhibiting effect in a concentration of 5 ng/g (group 7). At the same time, homologous defensin exerted a marked corticostatic effect even in a low dose, providing evidence of a partial species specificity of the phenomenon studied.

Thus, the data obtained suggest the presence of corticostatic properties in the defensin preparations tested with respect to the glucocorticoid responses caused in the organism of experimental animals by stress factors or corticotropin.

During stress the hormones have a significant effect on the formation of the organism's defense

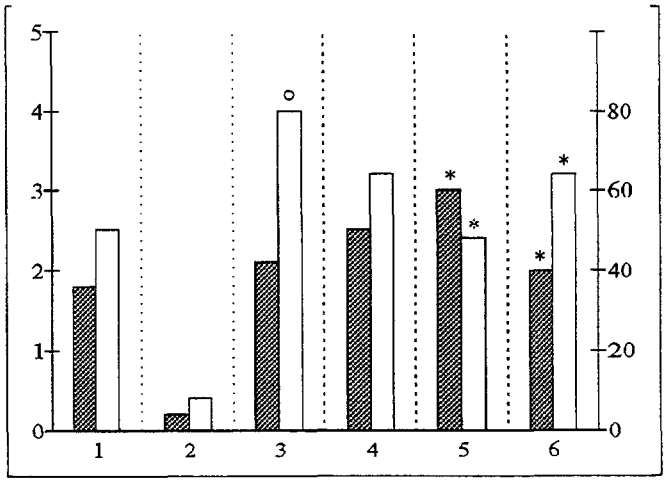

Fig. 3. Effect of defensins on stress-induced immunosuppression in mice. Abscissa: animal groups. 1) immunized animals not subjected to stress; 2) animals subjected to combined stress before immunization; 3) unstressed animals which received rabbit defensin in a dose of 20 μg/g; 4) unstressed animals which received rabbit defensin in a dose of 2 μg/g; 5) animals subjected to combined stress before immunization, which received 20 μg/g rabbit defensin 10 min before stress; 6) animals subjected to combined stress before immunization which received 2 μg/g defensin 10 min before stress. Circle: $p<0.05$ vs. group 1; asterisk: $p<0.05$ vs. group 2. Hatched columns: $\log_2$AB titer; unhatched columns: APC/10^6 splenocytes.

response including the immmune response. Adrenal hyperproduction of glucocorticoids is known to be one of the causes of stress-determined dysfunctions of the immmune system [6,12]. Judging by the facts previously discussed, one may assume that preventively administering defensins may offset the immunosuppressive effect of some stress factors. In fact, it was shown on the model of combined stress, which practically blocked the immune response (Fig, 3, group 2), that preliminarily injecting polypeptides in mice impaired the humoral immune response in the experimental animals (groups 5, 6). It should be mentioned that under these conditions defensins not only act as corticostatics, but are obviously capable of modulating the immune response (groups 3, 4) by means of other unknown mechanisms as well.

In the present study the ability of cationic polypeptides-defensins to reduce the stress- and ACTH-induced glucocorticoid response and to offset distress-determined immunosuppression in experimental animals has been demonstrated. These data speak in favor of the supposed corticostatic function of defensins [8,15] which has previously been found in culture. One may assume that the adaptive significance of the known phenomenon of the mobilization of neutrophils from the bone marrow (neutrophilia) [3] is not confined to preventive strengthening of the antimicrobial barrier of the organism. As neutrophils enter the blood and subsequently migrate into the tissues, a constant secretion of physiologically active compounds (including defensins) takes place in the granular apparatus of the neutrophils [14]. Here, the defensins, acting as humoral factors, exhibit their corticostatic and immunoprotective properties by providing for an interaction between the immune and neuroendocrine systems directed toward the formation of adaptive protective responses during stress.

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