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Immunotropic Effects of Active Immunization with Serotonin-Protein Conjugate in C57Bl/6 Mice Exposed to Immobilization Stress

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Immobilization stress for 14 h induced lymphopenia, hypoplasia of the thymus and spleen, and decrease in functional activity of T lymphocytes and macrophages in C57Bl/6 mice. Active induction of antibodies against serotonin during stress decreased functional activity of T lymphocytes and partially recovered phagocytic activity of peritoneal macrophages.

Key Words: *antiserotonin antibodies; stress; lymphocytes; macrophages*

Active induction of antiserotonin antibodies in experimental animals reduces alcohol consumption (by several times) and relieves symptoms of alcohol and morphine withdrawal [4-6]. Systemic administration of antibodies against serotonin (5-HT) in a dose of 25 mg/kg suppresses lymphocyte proliferation in response to pokeweed mitogens (PM) and concanavalin A (Con A) and increases phagocytic activity of peritoneal macrophages. However, addition of these antibodies in a dose of 10^{-7} M to cultured cells inhibits the blastogenic response of PM-stimulated lymphocytes and increases phagocytic activity of macrophages [1]. The effect of antiserotonin antibodies on the immune system during secondary immunodeficiency is of considerable interest. Immobilization stress produces an adverse effect on the immune system [3]. Here we studied the influence of immunization with the 5-HT-protein conjugate on some parameters of the immune system in C57Bl/6 mice subjected to immobilization stress. We measured the weights of lymphoid organs, estimated the count of peripheral blood leukocytes and lymphocytes, studied the proliferative response of Con A- and PM-stimulated lymphocytes , and determined phagocytic activity of peritoneal macrophages.

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MATERIALS AND METHODS

Experiments were performed on 50 male C57Bl/6 mice weighing 20-22 g. Antiserotonin antibodies were induced by active immunization with 5-HT-bovine serum albumin (BSA) synthesized as described elsewhere [2]. Immunization was performed as follows: first immunization with 5-HT-BSA (2 mg/kg) and complete Freund's adjuvant (CFA, Serva) subcutaneously in 2 points on the back; second immunization with 5 mg/kg 5-HT-BSA conjugate in 0.2 ml physiological saline intraperitoneally after 2 weeks (without CFA). The third immunization was performed with 5-HT-BSA (10 mg/kg intraperitoneally, without CFA) after 2 weeks. Control animals received an equivalent volume of physiological saline containing or not containing CFA. The antibodies against 5-HT in the plasma were assayed by enzyme-linked immunosorbent assay on a Mini-Reader device (Dynateck). A conjugate on heterologous protein carrier (5-HT and horse γ-globulin) served as the test antigen.

Experimental stress was modeled 1 week after the last immunization. The mice were immobilized in

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TABLE 1. Effect of Stress and Immunization with 5-HT-BSA Conjugate on the Weight of Lymphoid Organs and Count of Peripheral Blood Leukocytes and Lymphocytes in C57Bl/6 Mice (*M*±*m*)

Group	Thymus (% of body weight)	Spleen (% of body weight)	Leukocytes, thousands/mm^3	Lymphocytes	
				%	thousands/mm^3
Control	0.26±0.02	0.38±0.02	5.6±0.4	67.5±4.8	3.8±0.4
Stress, *n*=7	0.19±0.01***	0.30±0.02***	3.8±0.4***	35.0±4.2*	1.4±0.3*
Immunization with 5-HT-BSA and stress, *n*=7	0.14±0.02**	0.31±0.01**	4.2±0.4***	30.0±6.1*	1.2±0.2**
CFA and stress, *n*=6	0.19±0.01***	0.29±0.02***	4.3±0.4***	38.3±5.4**	1.6±0.2*
Immunization with 5-HT-BSA, *n*=7	0.22±0.02	0.39±0.04	7.9±1.1	51.6±7.0	4.4±1.3
CFA, *n*=6	0.20±0.01	0.34±0.02	7.1±0.7	38.3±6.0**	2.8±0.5

Note. Experimental conditions are shown in parentheses. *n*, number of animals. Here and in Table 2: *p<0.001, **p<0.01, and ***p<0.05 compared to the control.

tight closed tubes for 14 h. They were divided into 6 groups. Group 1-3 mice were subjected and group 4-6 mice were not subjected to experimental stress. Group 1 included intact mice. Group 2 and 4 mice were immunized with 5-HT-BSA conjugate. Group 3 and 5 mice received CFA during the first immunization, and group 6 mice received physiological saline (control).

One hour after stress the animals were killed under ether anesthesia. The thymus and spleen were removed and weighed, the count of blood leukocytes and lymphocytes was estimated routinely. For evaluation of phagocytic activity of macrophages peritoneal cells were washed out with medium 199, resuspended, placed in tubes with cover glasses, and incubated at 37°C for 1 h. After adhesion of macrophages to cover glasses the culture medium was replaced with a fresh portion containing *Staphylococcus aureus* strain Zhaev (5×10^6 cells, 24-h culture). Incubation was performed for 45 min. Glasses were removed and washed with physiological saline. Adherent cells were fixed with methanol and stained with azure and eosin (Romanovsky method). Phagocytic activity was determined by the percent of phagocytizing cells (phagocytic number) and number of bacteria engulfed by 1 phagocyte (phagocytic index). The reaction of blast transformation was performed with mouse spleen lymphocytes. The cells (2×10^6) were cultured in RPMI-1640 medium (Serva) containing 10% fetal bovine serum (Serva), antibiotics, and mitogen (Con A or PM). The volume of each sample was 2 ml. Mitogens (Serva) were applied in a dose of 20 μg per 10^6 cells. Our previous studies showed that mitogens in this dose produce the maximum blast transformation effect. ^{3}H-Thymidine (1 μCi) was added to samples 6 h before the end of culturing. Specific activity was measured on an Intertechnique device.

The results were analyzed by Student's *t* test (PRIMER software).

RESULTS

Immunization of C57Bl/6 mice with 5-HT-BSA conjugate induced production of antibodies against 5-HT in high titers (1:64). Immobilization stress produced leukopenia, lymphopenia, and atrophy of the thymus and spleen (Table 1). Preimmunization with 5-HT-BSA conjugate did not prevent the development of stress-induced pathological changes in immunocompetent organs, peripheral blood leukocytes, and lymphocytes. CFA had no effect on stress-induced changes in immunocompetent organs. Immunization with 5-HT-BSA conjugate and administration of CFA did not affect the weights of the thymus and spleen and counts of peripheral blood leukocytes and lymphocytes in C57Bl/6 mice (compared to control animals). Immobilization stress suppressed phagocytic activity of peritoneal macrophages, which was confirmed by the decrease in the phagocytic number and phagocytic index (Table 2). Active induction of antibodies against 5-HT led to partial recovery of phagocytic activity of

TABLE 2. Effect of Stress and Immunization with 5-HT-BSA Conjugate on Phagocytic Activity of Peritoneal Macrophages in C57Bl/6 Mice (*M*±*m*)

Group	Phagocytic number, %	Phagocytic index
Control	51.6±1.7	3.6±0.1
Stress	35.3±1.7*	3.2±0.1***
Immunization with 5-HT-BSA and stress	44.3±1.7*$^{+}$	3.6±0.1^{++}
CFA and stress	51.6±1.7^{++}	4.0±0.1***$^{++}$
Immunization with 5-HT-BSA	68.5±3.5**	4.5±0.2**
CFA	59.1±2.6***	4.1±0.3

Note. ^{+}p<0.01 and ^{++}p<0.05 compared to stress.

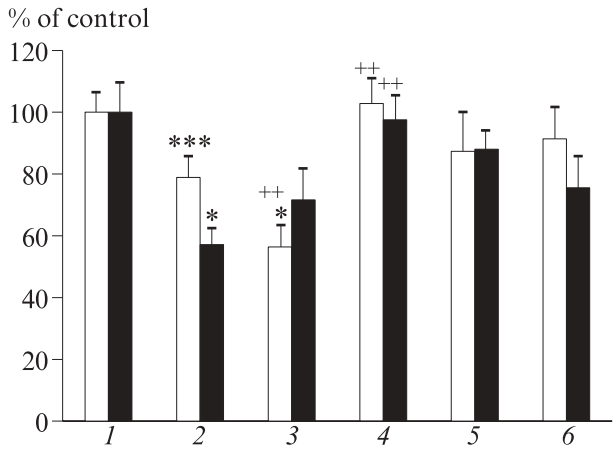

Fig. 1. Effect of stress and immunization with 5-HT-BSA conjugate on blast transformation of lymphocytes stimulated with concanavalin A (light bars) and pokeweed mitogen (dark bars). Control (*1*), stress (*2*), immunization with 5-HT-BSA and stress (*3*), CFA and stress (*4*), immunization with 5-HT-BSA (*5*), and CFA (*6*).

mouse peritoneal macrophages. Immunization with 5-HT-BSA conjugate and treatment with CFA increased phagocytic activity of peritoneal macrophages. CFA abolished stress-induced changes in phagocytosis of peritoneal macrophages; under these conditions phagocytic activity of cells surpassed the baseline level.

Immobilization stress suppressed the cell-mediated immune response (Fig. 1). Preimmunization with 5-HT-BSA conjugate potentiated the effect of stress on Con A-induced blast transformation of lymphocytes and insignificantly normalized the proliferative response of cells to PM. Pretreatment with CFA abolished the adverse effect of stress on the proliferative response of lymphocytes stimulated with Con A and PM. Immunization with 5-HT-BSA conjugate and administration of CFA had no effect on the blastogenic response of lymphocytes to Con A and PM.

Our findings suggest that preimmunization with 5-HT-BSA conjugate produces a complex effect on the immune system during immobilization stress. Active induction of antibodies against 5-HT significantly inhibited the proliferative response and was accompanied by partial recovery of phagocytic activity of peritoneal macrophages. Pretreatment with CFA in immobilization stress normalized the proliferative response of lymphocytes to Con A and PM and completely restored phagocytic activity of peritoneal macrophages. Previous studies showed that antibodies against 5-HT directly affect receptors on macrophages, which coincides with dose-dependent effect of 5-HT [1]. Moreover, 5-HT increases phagocytic activity of peritoneal macrophages. The effect of CFA stimulating phagocytic activity of peritoneal macrophages cannot be ignored. Inhibition of specific immune reactions during stress and active induction of antibodies against 5-HT can be explained by the state of neurotransmitter balance in the brain of C57Bl/6 mice. In these animals, serotoninergic influences dominate over dopaminergic influences [8]. The serotoninergic system blocks immune reactions, which is manifested in suppression of the immune response, inhibition of mitogen-induced blast transformation of lymphocytes, and decrease in killer activity of lymphocytes [3]. Our assumption is confirmed by published data that this method for immunization and induction of antiserotonin antibodies does not decrease 5-HT content in the brain of C57Bl/6 mice [7].

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