PREVENTION OF DEPRESSION OF NORMAL KILLER CELL ACTIVITY IN STRESS BY ADAPTATION TO PERIODIC HYPOXIA

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KEY WORDS: adaptation to hypoxia; normal killer cells; stress; DNA synthesis.

Preliminary adaptation to periodic hypoxia in a pressure chamber is known to increase the resistance of animals to stress-induced injury, and in particular, to prevent stress injuries of the heart [2, 6]. Meanwhile the problem of the protective effect of this kind of adaptation to periodic hypoxia during stress-induced disturbances of immunogenesis and, in particular, in the stress-induced depression of normal killer cell (NKC) activity demonstrated in recent years [4, 5, 9], remains unsolved. We know that adaptation to the periodic action of very considerable hypoxia ("ascent" in a pressure chamber to an altitude of 9000 m) leads to marked adaptation of DNA synthesis in thymus cells [8]. The possibility thus cannot be ruled out that gradual adaptation to moderate periodic hypoxia may somehow or other prevent depression of NKC activity, which plays an important role in antitumor resistance [7, 10].

The aim of this investigation was to determine the effect of preliminary adaptation to periodic hypoxia on stress-induced depression of NKC activity and also on DNA synthesis in thymus and spleen cells.

EXPERIMENTAL METHOD

Inbred male BALB/c mice weighing 16-18 g, from the "Svetlye Gory" Nursery, Academy of Medical Sciences of the USSR, were used.

The animals were adapted to periodic hypoxia in a pressure chamber for 6 h daily for 28 days; the mice spent the rest of the time in the animal house. The adaptation schedule was as follows: days 1-3 of adaptation: "ascent" to an altitude of 500-1000 m, days 3-6 — 1500 m; for the remaining 21 days the animals were adapted to an altitude of 2000 m.

All the animals were divided into four groups: 1) control intact mice, 2) animals adapted for 28 days to periodic hypoxia, 3) intact mice exposed to immobilization stress, 4) mice adapted to periodic hypoxia and then exposed to immobilization stress. Immobilization stress was produced by fixing the animals lying in the supine position for 6 h. The mice were decapitated 24 h after the end of exposure to stress and suspensions of thymocytes and splenocytes prepared.

NKC activity was determined by the test of radioactive chromium release from labeled target cells (these were YAC-1 mouse lymphoma cells transplanted *in vitro*) during incubation for 4 h with effector cells (mouse splenocytes). Full details of the technique were described previously [3]. The ability of spleen and thymus cells to synthesize DNA was determined by incubating them in a concentration of 2×10^6/ml in medium RPMI 1640 with 10% embryonic calf serum and 1% glutamine in the presence of [^{3}H]thymidine (5 μCi/ml) for 1 h. Radioactivity incorporated into the acid-insoluble fraction was estimated on a scintillation counter.

The significance of the results was determined by Student's test.

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TABLE 1. Effect of Adaptation to Periodic Hypoxia on Depression of NKC Activity (in %) in Immobilization Stress

Group of animals	Ratio of effector cells to target cells		
	100:1	50:1	25:1
1	28,3±2,2	20,0±1,9	14,4±1,7
2	25,3±2,4	21,7±2,1	16,3±1,8
3	15,0±1,6	12,3±1,3	9,4±1,0
4	22,1±2,1	20,3±2,0	15,2±1,4
P_{1-2}	>0,05	>0,05	>0,05
P_{1-3}	<0,05	<0,05	<0,05
P_{3-4}	<0,05	<0,05	<0,05

TABLE 2. Effect of Adaptation to Periodic Hypoxia on Stress-Induced Depression of DNA Synthesis in Thymus and Spleen Cells

Group of animals	Incorporation of [^{3}H]thymidine, cpm	
	splenocytes	thymocytes
1-	27 505±2 234	10 338±956
2-	29 116±2 712	16 903±1 451
3-	19 760±1 739	5 056±438
4-	30 601±2 587	17 629±1 530
P_{1-2}	>0,05	<0,05
P_{1-3}	<0,05	<0,05
P_{3-4}	<0,05	<0,05

Legend. Here and in Table 2, each point corresponds to 12 mice.

EXPERIMENTAL RESULTS

Under the influence of adaptation to periodic hypoxia under the conditions used no changes took place in NKC activity (Table 1). Meanwhile, stress-induced depression of activity of immunocompetent cells of this type against the background of the adaptation schedule used, as is well known from previous publications, was completely absent. Adaptation prevented the stress-induced depression of activity of calls of the natural cytotoxicity system.

In the second stage of the investigation an attempt was made to determine the effect of stress and of preliminary adaptation to hypoxia on DNA synthesis in thymocytes and splenocytes, for with the method used, this approximately reflected the number of cells in each organ capable of realizing DNA synthesis and, consequently, of proliferating. It was found that adaptation to hypoxia itself caused a tendency for DNA synthesis to increase in the splenocytes, and DNA synthesis increased much more in the thymocytes. When these data are interpreted, the fact established previously, that prolonged gradual adaptation to the peripheral action of hypoxia under pressure chamber conditions regularly causes a significant increase in weight of the spleen and in the number of splenocytes but, conversely, causes reduction of the weight of the thymus and of the number of thymocytes [1], must be borne in mind. It can accordingly be understood that even a small increase in the relative number of DNA-synthesizing splenocytes may signify a considerable increase in DNA synthesis for the organ as a whole. Conversely, even a large increase in the relative number of DNA-synthesizing cells in the thymus may signify a comparatively small increase in DNA synthesis in the whole organ. The main conclusion which can be drawn from the data in Table 2 is that stress causes significant inhibition of DNA synthesis in the cells of both immunocompetent organs studied, whereas preliminary adaptation to hypoxia completely prevents this phenomenon.

Preliminary adaptation to hypoxia of gradually increasing intensity under pressure chamber conditions thus undoubtedly is a factor which can effectively prevent stress-induced depression of DNA synthesis in the cells of immunocompetent organs and depression of NKC activity. The mechanism of this protective effect may be asscіated both with the central action of adaptation, reducing the magnitude of the stress response, and with its effect on immunocompetent organs. This problem requires further study.

LITERATURE CITED

1. F. Z. Meerson, G. T. Sukhikh, and B. A. Frolov, Immunologiya, No. 3, 34 (1981).
2. F. Z. Meerson and A. I. Saulya, Patol. Fiziol., No. 6, 50 (1982).
3. G. T. Sukhikh, F. Z. Meerson, L. V. Van'ko, et al., Vestn. Akad. Med. Nauk SSSR, No. 3, 16 (1983).
4. G. T. Sukhikh and F. Z. Meerson, Byull. Éksp. Biol. Med., No. 11, 84 (1983).
5. G. T. Sukhikh, in: Interaction between Normal Killer and Tumor Cells [in Russian], Moscow (1983), pp. 70-79.
6. A. A. Ugolev, L. Yu. Golubeva, and F. Z. Meerson, Kardiologiya, No. 11, 91 (1980).
7. B. B. Fuks, in: Interaction between Normal Killer and Tumor Cells [in Russian], Moscow (1983), pp. 5-26.

8. H. J. Aarstad et al., Scand. J. Immunol., 18, 461 (1983).
9. R. B. Herberman and J. R. Ortaldo, Science, 214, 24 (1981).

RHESUS-LIKE ANTIGENIC ACTIVITY MANIFESTED IN RED BLOOD CELLS OF RHESUS-NEGATIVE BLOOD DONORS AND INCREASED EXPRESSION OF ABO ANTIGENS AFTER UV-IRRADIATION OF BLOOD

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The writers showed previously that UV irradiation causes partial destruction of the outer juxtamembranous layer in autonomous mammalian tissue cells [2, 3, 6, 7]. In lymphocytes this is accompanied by changes in expression of membrane receptors [4, 5].

The aim of the present investigation was to discover whether expression of membrane antigens of the ABO and rhesus systems in human red blood cells (RBCs) is modified by UV irradiation.

EXPERIMENTAL METHOD

RBCs were obtained from freshly prepared packed red cells from blood obtained from 23 donors of different groups, stabilized with "Glyugitsir" solution. There were three series of experiments: I) on isolated RBCs washed three times with buffered NaCl solution (0.9%) and resuspended in it in a concentration of 5×10^7 cells/ml; II) on RBCs from packed cells diluted to a concentration of 5×10^7 cells/ml with solution TsOLIPK No. 8b; III) on RBCs from undiluted (intact) packed red cells. UV irradiation (254 nm) was carried out by DB-30 tubes in doses which increased hemolysis of RBCs by 5-32% after 2-3 h. Agglutinating activity of antigens of the ABO and rhesus systems was investigated in accordance with current instructions, using standard isohemagglutinating sera. The degree of agglutination of RBCs was estimated by microscopy on a 4-point system (4 points denotes the maximal reaction).

EXPERIMENTAL RESULTS

Agglutinating activity of antigens of the ABO system of unirradiated isolated RBCs from different donors varied: The minimal concentration of antibodies in which RBCs began to agglutinate was 1:64 for some blood samples and 1:8 for others. Antigens of the ABO system 2-3 h after irradiation began to be detected in the presence of a lower concentration of antibodies in the serum: antigens A and B in a titer of 1:128, H antigen in a titer of 1:32 (Table 1); this indicated an increase in agglutinating activity of the antigens by 2-4 times. The stimulating action of irradiation was exhibited in all blood samples tested, but was strongest in samples with the lowest initial antigenic activity (Table 1); in these cases their expression was increased by 8-16 times. The effect decreased appreciably after 24 h. Similar results were obtained when RBCs from diluted and intact packed cells were subjected to UV irradiation, but the effect of irradiation in this case was still present 24 h after exposure. The explanation for this could be that irradiated RBCs survive better in the form of packed cells than when diluted in physiological saline.

An increase in agglutinating activity of the $Rh_0(D)$ antigen of the rhesus system was found in RBCs of only one of six samples of RH^+ blood tested (Table 1) which was distinguished by the low initial activity of this antigen (1:32) compared with the rest (1:512). A stimulating action of UV irradiation was recorded in this case in isolated RBCs and in RBCs of in-

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