EFFECT OF EXOGENOUS WHOLE-BODY HYPERTHERMIA ON THE STATE OF HUMORAL IMMUNITY

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A high ambient temperature is an extremal factor acting on the body. Man is exposed to hyperthermia either when unprotected against a high temperature under natural conditions, or in a specific industrial situation [1, 4]. Quite often the action of a high temperature on the human body calls for a study of the functional systems during hyperthermia and adaptation to heat, and the search for methods of increasing the resistance of the body under these conditions. The most important functional system of the body, responsible for maintaining its viability, is the immune system, but information on its response to hyperthermia in the literature is limited and contradictory. In recent years, moreover, whole-body exogenous hyperthermia has achieved widespread popularity in clinical practice and, in particular, in the combination treatment of cancer patients. However, the use of the heat factor in the treatment of cancer patients is often accompanied by whole-body hyperthermia. The study of the state of immunity during hyperthermia, which largely determines the results of treatment of cancer patients, is therefore a practical necessity [2, 5].

The aim of this investigation was to study some parameters of humoral immunity in acute hyperthermia and also during long-term, interrupted exposure of the body to a high ambient temperature.

EXPERIMENTAL METHOD

First-generation (CBA × C57BL/6)F_1 hybrid mice weighing 24-26 g were used in the experiments. Hyperthermia of the animals was produced in a hot chamber at a temperature of 43-44°C, with constant ventilation. Acute hyperthermia was reduced to a single exposure of the animals in the hot chamber until their rectal temperature reached 42°C, and to the stage of heat shock (rectal temperature 43-43.5°C). Long-term interrupted hyperthermia was produced in animals by keeping mice daily in the hot chamber for 20 min. The animals were exposed to heat on 3, 5, 10, 20, 30, and 40 days. The average rise of rectal temperature during the first period of hyperthermia was 42°C. To assess humoral immunity we studied the proliferative activity of spleen cells (SC) in the blast-transformation reaction of lymphocytes (BTRL) [3] in response to stimulation by polyclonal B-cell mitogens, Escherichia coli lipopolysaccharide (LPS) and pokeweed mitogen (PWM), and also the number of antibody-forming cells (AFC) in the spleen on the 5th day after injection of the antigen [6]. Sheep's red blood cells were used as the antigen and were injected intraperitoneally into the mice in a dose of $5 \cdot 10^8$ cells. Immunization of the animals exposed once to a temperature of 42°C took place after their removal from the chamber. In the case of chronic hyperthermia the antigen was injected 24 h after the appropriate session of hyperthermia. To study the effect of heat shock on the number of AFC the animals were heated at the peak of antibody formation. To investigate the proliferative response of SC to acute hyperthermia, material for testing was removed immediately after the animals were taken from the hot chamber, whereas with chronic hyperthermia, it was obtained 1 day after the last exposure to hyperthermia. When the BTRL test was set up, the cells were cultured in medium RPMI-1640, to which were added human serum (10%), L-glutamine (2 mM), 2-mercaptoethanoiamine ($5 \cdot 10^{-5}$ M), and HEPES buffer (10 mM). The cellular response

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TABLE 1. Proliferative Response of SC and Number of AFC in Acute Hyperthermia (M ± m)

Parameter	Series of experiments			
	hyperthermia up to a temp. of 42°C		hyperthermia to heat shock	
	control	experiment	control	experiment
Proliferative response				
LPS	3,07±0,34	3,82±0,54	4,94±0,55	1,13±0,17*
PWM	3,95±0,30	5,13±0,40*	7,33±0,69	1,35±0,16*
Number of AFC per 10^6 SC	433,6±22,3	462,8±40,1	383,0±56,7	209,1±23,9*

Legend. Here and in Table 2: data on proliferative activity are shown as stimulation indices, asterisk indicates that differences from control are significant.

TABLE 2. Proliferative Response of SC and Number of AFC in Chronic Hyperthermia (M ± m)

Parameter	Duration of exposure to heat, days											
	Series of experiments											
	3		5		10		20		30		40	
	control	expt.	control	expt.	control	expt.	control	expt.	control	expt.	control	expt.
Proliferative response												
LPS	2,82± 0,29	3,17± 0,30	3,17± 0,36	3,36± 0,35	3,71± 0,18	1,62± 0,15*	3,49± 0,28	2,29± 0,31*	3,41± 0,25	2,51± 0,24*	6,04± 0,83	4,63± 0,75
PWM	5,02± 0,33	6,29± 0,91	4,55± 0,58	4,89± 0,34	5,66± 0,46	3,80± 0,18*	5,33± 0,41	3,19± 0,26*	5,83± 0,37	5,01± 0,21*	4,79± 0,23	4,97± 0,72
Number of AFC per 10^6 SC	499,0± 29,3	579,8± 33,1	591,0± 48,5	317,0± 92,8*.	494,6± 21,6	367,6± 55,9*	570,4± 66,2	174,7± 38,1*	445,0± 29,7	431,6± 24,8	393,8± 25,6	420,8± 16,5

was recorded as incorporation of ^{3}H-thymidine into DNA of the proliferating cells, followed by calculation of the stimulation index (SI):

$$\text{ИС} = \frac{\text{cpm after stimulation}}{\text{cpm control}}.$$

A series of experiments consisted of 6-15 animals. Each variant of the BTRL was carried out in six tubes, and when AFC was determined, in two tubes. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

In animals exposed to acute hyperthermia, up to a rectal temperature of 42°C an increase in proliferative activity of SC was observed during stimulation with PWM (Table 1). The proliferative response of the spleen cells to stimulation by the lipopolysaccharide did not differ from that in the control. A single exposure to hyperthermia likewise had no effect on the number of AFC in the spleen. Overheating the mice to the stage of heat shock was accompanied by marked depression of the proliferative activity of SC in response to stimulation by mitogens and by a reduction in the number of AFC in the spleen, probably as a result of the direct cytotoxic action of a high temperature on lymphocytes.

During long-term exposure to a high ambient temperature the degree of change in the functional activity of the B lymphocytes depended on the duration of hyperthermia (Table 2). For instance, hyperthermia of the animals for 3 and 5 days was not accompanied by any change in proliferative activity of SC in response to stimulation both by lipopolysaccharide and by pokeweed mitogen. However, the number of AFC in the spleen of mice exposed to a high temperature for 5 days was reduced, possibly indicating disturbance of the mechanisms of differentiation of B lymphocytes while their proliferative capacity was preserved during exposure to a high temperature. The effect of the high temperature on the animals for 10 and 20 days was characterized by the development of marked immunosuppression, as shown by inhibition of the proliferative activity of SC in response to stimulation by mitogens and to an

increase in the number of AFC in the spleen. Heating the mice for 30 days was accompanied by restoration of the number of AFC in the spleen, while the proliferative response of SC to the mitogens remained depressed.

During chronic exposure to interrupted hyperthermia of the animals the immune response was restored as the animals became adapted to this type of exposure to heat, as shown by the return of proliferative activity of the spleen cells to normal in response to stimulation by B-cell mitogens and preservation of the number of AFC at the control level 40 days after the beginning of hyperthermia.

REFERENCES

1. N. B. Kozlov, Hyperthermia: Biochemical Basis of Its Pathogenesis, Prevention, and Treatment [in Russian], Voronezh (1990).
2. V. F. Lopatin, Med. Radiol., No. 7, 60 (1983).
3. V. V. Khorobrykh, L. V. Pronin, A. F. Kirkin, et al., Immunologiya, No. 3, 76 (1983).
4. S. M. Shablenko, Vrach. Delo, No. 2, 95 (1989).
5. M. Janiak and S. Szmigielski, Brit. J. Cancer, **45**, No. 5, 122 (1982).
6. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).

VITAMIN D ENDOCRINE SYSTEM AND BONE TISSUE MINERAL METABOLISM IN RATS WITH ADJUVANT ARTHRITIS: EFFECT OF 1,25-DIHYDROXYVITAMIN D_3

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Adjuvant arthritis in rats is an autoimmune disease induced by subcutaneous injection of mycobacteria in mineral oil, and it reflects reasonably adequately pathological changes developing in rheumatoid arthritis in man, and is widely used as an experimental model of this disease in the quest for antiinflammatory and antiarthritic agents. In the chronic stage of adjuvant arthritis intensive destruction of cartilage takes place, the content of collagen and calcium in the bones is reduced, and osteoporosis develops [7]. A basic role in the regulation of mineral metabolism in bone tissue is played by the vitamin D endocrine system. The most active form of vitamin D, and responsible for realization of its function in the body, is 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. It is now known that 1,25$(OH)_2D_3$ possesses marked immunomodulating activity [1]. In vivo 1,25$(OH)_2D_3$ functions through a hormonal mechanism, interacting in target tissues with specific receptors [5].

The aim of this investigation was to study the state of the vitamin D endocrine system of bone tissue and mineral metabolism in rats with adjuvant arthritis, and the effect of 1,25$(OH)_2D_3$ on these parameters.

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