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α1- and β-Adrenoblockers Effects on Immunogenesis in Rats under Thermoneutral Conditions and after Cooling of Various Extent

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Experiments with rats showed that ionophoretic delivery of α1-adrenoblocker into the skin does not change effects of superficial and deep cooling and did not affect the amount of the antibody producing cells in spleen and a blood level of circulating antibodies under thermoneutral conditions. Meanwhile α1-adrenoblocker abolished inhibitory effect of fast deep cooling on antigen-binding properties of spleen cells and peritoneal extraction cells, and markedly stimulated it. β-Adrenoblocker had no effect on immunogenesis under thermoneutral conditions and changes modulating effect of cooling on immune response. It abolished immunosuppressive effect of deep cooling and enhanced the stimulating effect of superficial cooling. It was true both for antigen-binding processes in spleen and peritoneal cavity and for antibody production in spleen. The results obtained indicate an involvement of α1- and β-adrenoreceptors in immune response inhibition after deep cooling. Adrenoblockers effects on various immune competent cells are ambiguous and depend on temperature.

Key Words: *immune response, cooling, β-adrenoblocker, α1-adrenoblocker*

Temperature is one of crucial factors for development of physiological processes in the organism. Evaluation of cooling effects on whole organism and on distinct organs is of doubtless relevance.

This study was performed to investigate interactions of thermal control, adrenergic and immune systems during direct or indirect reactions to cooling and klendusity development. Previously was shown that development of immune response is affected during temperature actions on organism [1,2,3,6], moreover, according to our results the extent and direction of immune response changes depend on cooling fashion (depth and velocity) [1]. Superficial cooling has stimulating and deep, alternatively, inhibitory effects on antigen-binding and antibody producing functions of spleen and peritoneal extraction immune competent cells.

Sympathetic nervous system activation and relevant mediator release are essential components of organism protection against cold. Possible norepinephrine effects on immune reactions were shown by number of authors [4,9]. In our studies were established norepinephrine-produced changes of immune effect, developed both in thermoneutral conditions and under exposure to subsequent cooling [8]. That gave an opportunity to suggest the

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modulatory effects of exposure to cold to be associated with changes in sympathetic nervous system activity during cooling. Since norepinephrine effect can be objectified through action to various receptors the current study was carried out to explore involvement of α1- and β-adrenoreceptors in immunomodulation during exposure to cold.

MATERIALS AND METHODS

Experiment was carried out on Wistar male rats (10 and more animals per group) at 23°C at room. 5×10^{-8} sheep erythrocytes in 0.5 ml of 0.9% NaCl solution were injected intraperitoneally as an antigen. At day 5 blood, peritoneal exudate and spleen were taken from decapitated animals to evaluate the immune reaction against antigen. Thereafter the number of antigen-binding (rosette-forming) cells (ABC) in 1000 viewed, amount of antibody producing (IgM) cells (APC) in spleen and amount of circulating antibodies by hemagglutinin (log2) serum titre were estimated using conventional methods [1].

For adrenoreceptor function assessment rats using local ionophoresis on preliminary cleared from hair abdomen skin area (5×5 sm) using device GE-5-05 at current intensity 0.08 mA/sm^2 in 20 min in conventional therapeutic concentrations received α1-adrenoblocker verapamil (2.5 mg/ml) and β-adrenoblocker obzidan (1 mg/ml).

At a baseline (before cooling) animal temperature was maintained using heating table (rectal temperature 36.30±0.16°C, skin temperature 37.40± 0.12°C). Abdomen skin area exposed to ionophoresis was cooled down using thermode at a velocity 0.05°C/sec (fast cooling) until rectal temperature lowering by 1°C (superficial cooling) or by 3-4°C (deep cooling). For cooling depth control the rectal and skin temperature was measured and registered using thermocouple and computer system Term.

To exclude emotional component all manipulations (ionophoresis, temperature sensor placement, cooling, immunization at the peak of cold action) were performed under anesthesia (Nembutal, 40 mg/kg).

For detection of immunomodulatory effects animals were proceeded through one of experimental schedules: anesthesia, immunization (control); anesthesia, adrenoblocker ionophoresis, immunization (adrenoblocker effects under thermoneutral conditions); anesthesia, cooling, immunization (effects of preliminary cooling on immunization); anesthesia, adrenoblocker ionophoresis, cooling, immunization (adrenoblocker effects on cooling action). Previously it was established, that ionophoretic delivery of distilled water (vehicle) does not affect immune response [8].

Statistical data analysis was carried out using Student's *t* test.

RESULTS

Similarly to our previous studies [1,8], fast cooling exposed with no effect to circulating antibodies in blood (hemagglutination) influenced the antigen-binding function of spleen and peritoneal exudates cells, and the antibody producing in spleen too: superficial cooling stimulated this processes, and deep cooling — suppressed. The lack of changes in amount of blood circulating antibodies in both cooling types may indicate the less sensitivity of this constituent of immune response to antigen in comparison to response to cooling.

α1-Receptor blockade by verapamil under thermoneutral conditions does not affects amount of APC in spleen and characteristics of hemagglutination, and had equivocal effects on ABC of various localization: ABC number increased in spleen, but decreased in peritoneal exudates (Figs. 1, 2).

Preliminary α1-adrenoblocker administration did not affected modulatory effects of superficial and deep cooling on APC in spleen and hemagglutinin blood level. Meanwhile α1-blocker effects on antigen binding pronounced under thermoneutral conditions, were also noted after cooling. On the background of α1-adrenoblocker action both superficial and deep cooling led to stimulation of antigen binding in spleen and peritoneal exudate, amount of ABC after exposure to cold significantly increases in 3-4 times after superficial cooling and in 6-7 times after deep cooling (Fig. 2). Taking into account the fact that superficial cooling in the absence of α1-adrenoblocker led to increase in ABC number only by 30-40%, and deep cooling led even to decrease in ABC number, it may be concluded that regularities of cooling effects on antigen-binding under influence of α1-adrenoblocker dramatically changed.

Mentioned results indicate that α1-receptors virtually not involved in modulation of antibody producing cells accumulation both under thermoneutral conditions and after cooling. At the same time they contribute significantly to antigen binding both in thermoneutral conditions and after inhibitory action of deep cooling on antigen-binding cell number.

The data is published about norepinephrine effects on immune processes produced partially via α-adrenoreceptors [4,9]. Our investigation showed that α1-adrenoblockator verapamil abolishes inhi-

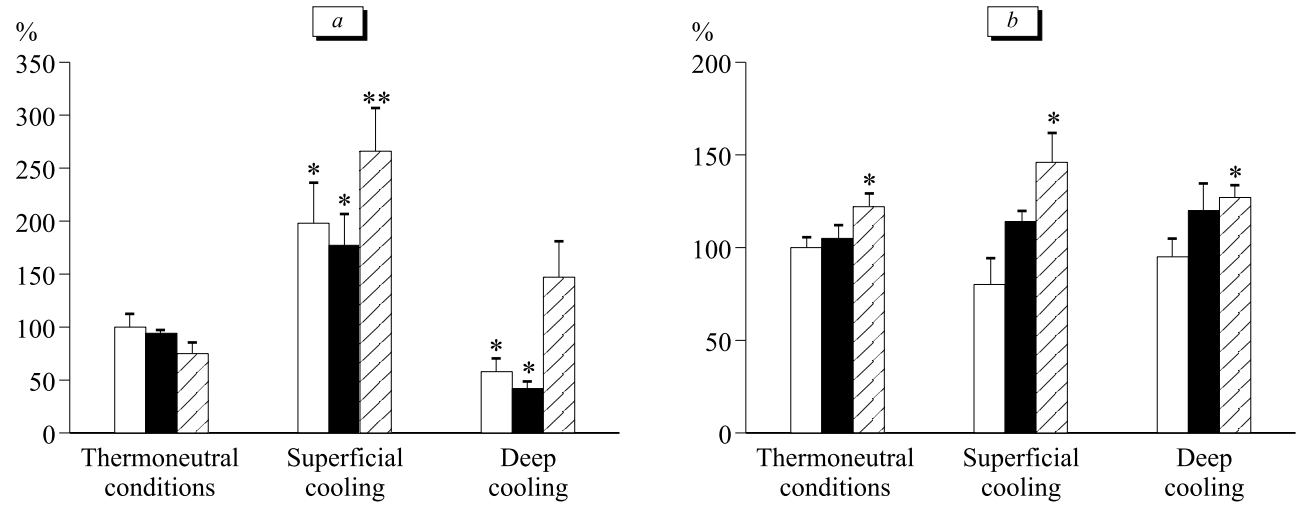

Fig. 1. Effects of α1- and β-adrenoblockers on APC amount in spleen (*a*) and on hemagglutinin blood level (*b*). Values obtained under thermoneutral conditions without blockers were considered for 100%. Here and at Fig. 2: light bars — without blockers, dark — verapamil, dashed — obzidan. **p*<0.05, ***p*<0.01 in comparison with the values obtained under thermoneutral conditions without blockers.

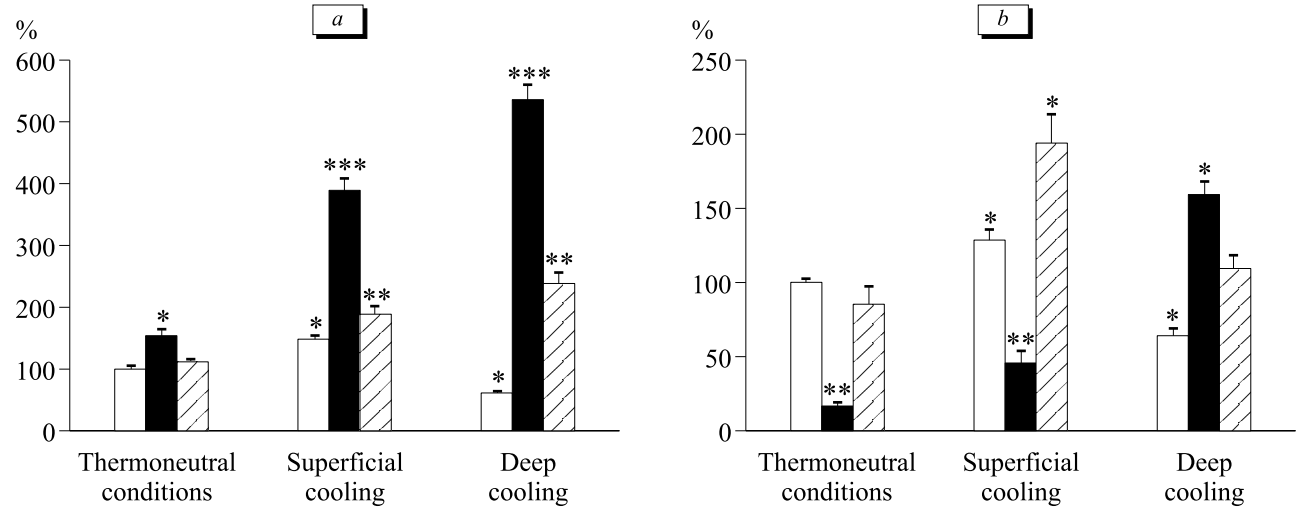

Fig. 2. Effects of α1- and β-adrenoblockers on ABC amount in spleen (*a*) and peritoneal exudates (*b*). Values obtained under thermoneutral conditions without blockers were considered for 100%: *a* — ABC amount per 1000 viewed in spleen; *b* — in peritoneal exudates. **p*<0.05, ***p*<0.01, ****p*<0.001 in comparison with the values obtained under thermoneutral conditions without blockers.

bitory effect of fast deep cooling on antigen-binding function of spleen cells. Experiments on mice revealed that α-adrenoblockator fentolamin has no effect on decrease in immune response after cooling stress [3]. Differences in effects may be associated with different types of animals, different roots of administration (systemic or ionophoretic through skin) and also with the fact that fentolamin is nonselective blockator whereas verapamil blocks only α1-adrenoreceptors, but not α2-adrenoreceptors.

Under thermoneutral condition ionophoretic delivery of β-adrenoblocker obzidan through skin had no effect on both antibody producing (Fig. 1) and antigen-binding functions of immune-competent cells in spleen (Fig. 2), the characteristics of antigen-binding of peritoneal exudate cells were also not affected (Fig. 2). β-Adrenoblocker administration under thermoneutral conditions, unlike the α1-adrenoblocker administration, led to increase (20%) in blood circulating antibodies.

Superficial cooling on the background of β-adrenoblocker administration was associated with more pronounced stimulation of both antigen-binding and antibody producing in spleen in comparison with that cooling schedule in the absence of β-adrenoblocker (Fig. 1, 2).

Fast deep cooling was associated with immune response suppression (both antigen-binding and antibody production). After deep fast cooling on

the background of β-adrenoblocker administration the immunosuppressive effect was abolished and even an immune response stimulation was noted (Fig. 2).

Antigen-binding changes in peritoneal exudates cells under exposure to β-adrenoblocker were similar to those, noted in spleen cells (Fig. 2).

Preservation of superficial cooling immunostimulating effect on the background of β-adrenoblocker administration and abolishment of deep cooling immunosuppressive effects allow to suggest that β-adrenoreceptors are involved in mechanisms of immune response suppression, produced by deep fast cooling.

Exposure to cold on the background of β-adrenoblocker and without it was not associated with additional changes in hemagglutination characteristics, and it remained to be elevated in comparison with intact animals. Increase in blood circulating antibody level at obzidan administration indicates the β-adrenoreceptor involvement in regulation of this immune parameter. All this results indicate an equivocal β-adrenoblocker effects on various immune competent cells and a relationship of this effects and thermal conditions.

Results of this investigation and published studies allow expressing of some assumptions concerning sources and mechanisms of noted modulation of immune response under conditions of cold. As it was previously mentioned, exposure to cold is associated with sympathetic nervous system stimulation, what may cause the inhibition of number of immune system functions [9]. However, recently appeared studies, which show that not all immune system functions are unambiguously suppressed after sympathetic nervous system stimulation [10]. To date, however, there are some papers where a presumption was made concerning selective inhibition of Th1-response and stimulation of Th2-response by catecholamines, but not concerning total immunosuppression [4]. This processes were showed to involve β2-adrenoreceptors: β2-adrenoreceptor agonists suppressed development of Th1-cells, promoting Th2-helpers differentiation [11].

As the deep cooling changes the superficial one, when besides skin receptors the deep ones involve, norepinephrine and epinephrine blood levels increase [7]. Direction of immune response changes (induction or suppression) probably depends on sympathoadrenal system activation rate. Intense activation after deep cooling may provide inhibitory action n immune response. Objectification of this suppression, as this study shows, involves β-adrenoreceptors. Blockade of presinaptic β-adrenoreceptors is able to decrease norepinephrine release, thus abolishing deep cooling-produced immune response suppression.

Noncontractive thermogenesis is well established to develop during exposure to cold. In rats it provided mainly by brown adipose tissue with b-adrenoreceptor participation. Relationship between brown adipose tissue and immune system is not well established, but significant induction of cell-mediated immunity after removal of intercsapular brown adipose tissue in neonatal period is known [5], and inhibitory effects of brown adipose tissue protein extract on thymus development was also noted [12]. Noncontractive thermogenesis extenuation under β-adrenoblocker influence can be one of the reasons of deep cooling-produced immunosuppression abolishment. α1- and β-adrenoblocker administration may also affect migration processes of immune competent cells due to blood flow redistribution.

Thus, α1-adrenoblocker ionophoretic delivery into skin does not affect number of antibody producing (IgM) B-cells in spleen and circulating antibody blood level under thermoneutral conditions. At the same time, α1-adrenoblocker abolishes inhibitory effect of deep fast cooling on antigen-binding function of spleen and peritoneal exudate cells, and significantly induces it.

β-Adrenoreceptor administration has no effect on spleen and peritoneal exudate cell immune response under thermoneutral conditions, and affects modulatory influence of cold to immune response. Immunosuppressive effect of deep cooling is abolished and stimulatory effect of superficial cooling is potentiated. It is true for both an antigen-binding in spleen and peritoneal cavity and an antibody production in spleen.

The results obtained indicate the α1- and β-adrenoreceptor involvement in immune response suppression after deep cooling. Furthermore antibody production suppression in spleen cells involves β-adrenoreceptors, and the antigen-binding in spleen and peritoneal cells involves both β- and α1-adrenoreceptors. Possibility of α2-adrenoreceptor involvement is not clear and demands performance of additional researches.

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