Modulating Effects of Calcium on Immune Response of Homoiothermal Animal under Thermoneutral Conditions and during Deep Cooling

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Preliminary ionophoretic administration of Ca^{2+} ions into the skin prevents the inhibitory effects of deep cooling on some processes characterizing the immune response. Differently directed changes in some immune response parameters induced by exogenous calcium and deep cooling suggest that competitive interactions between calcium-dependent processes can serve as mechanisms of functional changes in various physiological systems during the formation of the systemic reaction of a homoiothermal organism to cold.

Key Words: *immune response; cold; calcium*

The mechanisms regulating the formation of various effector reactions of the body to thermal influences are a fundamental problem of biology. Under these conditions, thermal signal is a trigging and forming factor; it regulates activity and cooperation between different physiological and intracellular systems. Our previous experiments demonstrated the role of thermal factor in the development of not only thermoregulatory processes, but also systemic immune response. Cold exposure modulates the development of the immune response and these changes depended on the rate and degree of cooling [1,6,7]. These observations agree with previous findings [3-5].

Taking into account the involvement of Ca^{2+} ions into activation of sympathetic nervous system participating in the formation of systemic reactions to cold, the key role of these ions in activation of peripheral thermoreceptors, and the key role of Ca^{2+} ions in the formation of systemic immune response, especially at the initial stages, we hypothesized that additional administration of calcium can affect the formation of the immune response during cooling. This assumption is also substantiated by the fact that cold exposure can decrease the concentrations of Ca^{2+} and calcitonin in the blood.

Here we studied the modulating effect of Ca^{2+} ions of the formation of some parameters of the immune response to thymus-dependent antigen under thermoneutral conditions and during deep cooling.

MATERIALS AND METHODS

Experiment was carried out on male Wistar rats (10 and more animals per group) at ambient temperature of 23°C.

Ca^{2+} ions were administered to animals under thermoneutral conditions by local ionophoresis on shaven abdominal skin (5×5 cm) using a GE-5-05 device at current intensity of 0.08 mA/cm^2 over 20 min. Cold stimulus was then applied to the same skin area.

The initial body temperature (before cooling) was maintained using a heating table (rectal temperature 36.30±0.16°C, skin temperature 37.40±0.12°C). Cold exposure was provided using a thermode at a rate 0.05°C/sec (fast cooling) or 0.005°C/sec (slow cooling) until rectal temperature drop by 3-4°C. For controlling cooling depth, the rectal and skin temperatures

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were measured and recorded using a thermocouple and Biopac system.

After the core temperature dropped by 3-4°C, the animals were intraperitoneally immunized with 5×10^{-8} sheep erythrocytes in 0.5 ml 0.9% NaCl and then warmed. On day 5, the blood, peritoneal exudate, and spleen were taken from decapitated animals for evaluation of the immune reaction to the antigen. To this end, the antigen-binding function (by the number of rosette-forming cells in the spleen and peritoneal lavage), the antibody-producing function (by number of IgM-producing cells in the spleen and by amount of hemagglutinin in the serum) were evaluated as described elsewhere [1].

To exclude emotional component, all manipulations (mounting of temperature sensors, ionophoresis, cooling, and immunization at the peak of cold exposure) were performed under anesthesia (nembutal, 40 mg/kg).

For detection of the immunomodulatory effects, the animals were proceeded through one of experimental schedules: anesthesia+immunization (scheme 1, control); anesthesia+calcium ionophoresis+immunization (scheme 2, calcium effects under thermoneutral conditions); anesthesia+cooling+immunization (scheme 3, effects of preliminary cooling on immunization); anesthesia+calcium ionophoresis+cooling+immunization (scheme 4, modulation of cooling effects with calcium ions). It was previously established, that ionophoretic application of distilled water (vehicle) does not affect the immune response [10].

The data were processed statistically using Student's *t* test; the results are presented on figures as $M\pm m$.

RESULTS

Under thermoneutral conditions without calcium ion administration, the number of rosette-forming cells in the spleen and peritoneal exudate was 50.0±1.2 and 36.0±1.0 per 1000 cells, respectively, the number of antibody IgM-producing splenic cells was 776,537± 135,155, and serum level of hemagglutinin (hemagglutinin titre/log2) was 5.30±0.15.

Under baseline condition (without cooling), administration of Ca^{2+} sharply increased antigen binding in the spleen and peritoneal exudate (Fig. 1); the number of antigen-binding cells in the spleen and peritoneal exudate increased 2.5-2.0-fold. Changes in number of IgM-producing cells in the spleen after Ca^{2+} ionophoresis were insignificant, although a trend forward intensification of antibody production was noted; the content of hemagglutinins in the blood remained unchanged.

Published data on the effects of Ca^{2+} ions on the immune process suggest that lymphocyte activation under certain conditions critically depends on calcium entry into the cell, and that calcium channel blockers induce immunodepression [11]. Our results suggest that Ca^{2+} ions primarily modulate the antigen binding processes.

Cold exposure (in both fast and slow regimens) before immunization suppressed antigen binding in the spleen and peritoneal exudates (Fig. 2); antibody production in the spleen was also suppressed after both fast and slow cooling, while the decrease in blood hemagglutinin level was noted only after slow cooling. These results confirm our previous findings [1,9].

The use of various cooling regimens characterized by the presence (fast cooling) or absence (slow cooling) of dynamic activity of skin cold receptors showed that slow deep cooling produced a more pronounced suppressive action on the immune response compared to that of fast deep cooling. This suggests that dynamic activity of cold receptors of the skin counteracts the suppressive action of deep cooling on the immune response providing different activation of the sympathoadrenal system, and hence, different catecholamine concentrations in the blood [8].

Preliminary ionophoretic application of Ca^{2+} ions counteracts the suppressive effects of deep cooling on some processes characterizing the immune response (Fig. 3). The suppressive effects of fast deep cooling on antigen-binding function of spleen cells become less pronounced, but suppression of antigen-binding by peritoneal immune cells remains virtually unchanged. The antibody production by splenic cells was not suppressed after deep cooling and even increased after fast deep cooling. Deep slow cooling did not

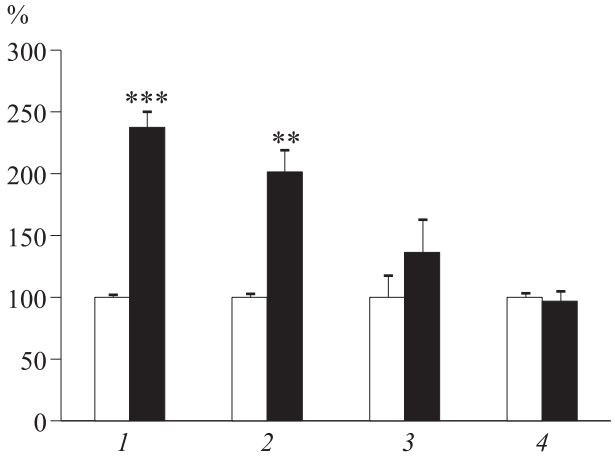

Fig. 1. Effects of ionophoretic calcium administration (dark bars) on parameters of the immune response under thermoneutral conditions. Here and on Fig. 2: parameters of the immune response under thermoneutral conditions without application of Ca^{2+} ions (light bars) were taken as 100%. Here and on Figs. 2 and 3: *1*) rosette-forming splenic cells; *2*) rosette-forming cells from peritoneal exudate; *3*) antibody producing splenic cells; *4*) hemagglutinin. ***$p<0.001$ and **$p<0.01$ compared to values without Ca^{2+} ions.

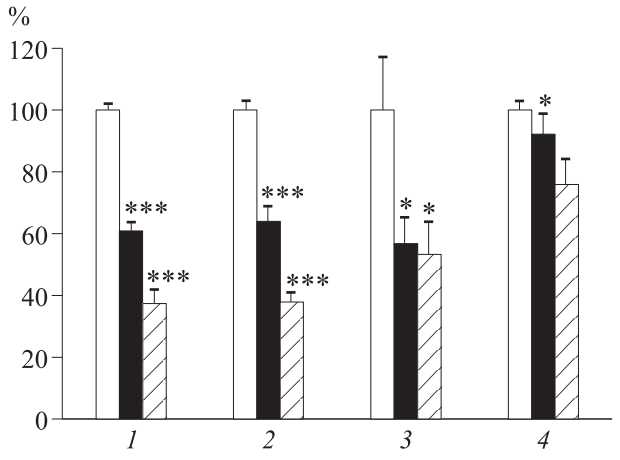

Fig. 2. Effects of fast (dark bars) and slow (shaded bars) deep cooling without application of Ca^{2+} ions on immune response parameters. *** $p<0.001$ and * $p<0.05$ compared to thermoneutral conditions.

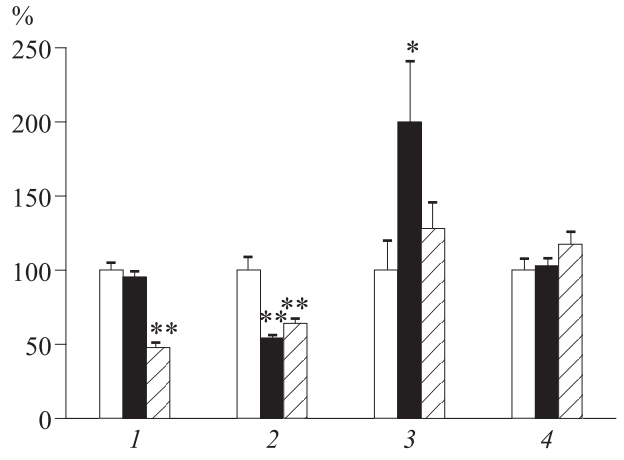

Fig. 3. Changes in parameters of the immune response after fast (dark bars) and slow (shaded bars) deep cooling after preliminary application of Ca^{2+} ions. Parameters of immune response under thermoneutral conditions after Ca^{2+} ion application (light bars) were taken as 100%. ** $p<0.01$ and * $p<0.05$ compared to thermoneutral conditions.

suppress blood level of hemagglutinins after calcium ionophoresis. These findings also demonstrate more pronounced suppressive effects of slow cooling not associated with dynamic activity of skin cold receptors on parameters of the immune response.

Attenuation of the suppressive effects of deep cooling after ionophoretic application of Ca^{2+} ions can be explained on the basis of our previous data on the role of different adrenoreceptors in modulation of the immune response during cold exposure. The suppressive effect of deep cooling is associated with involvement of β-adrenoreceptors, whereas the stimulating effect of mild cooling on the immune response involves $\alpha_{1,2}$-adrenoreceptors [2,7]. Calcium is a second messenger of α-adrenoreceptors, and the increase in calcium concentration promotes activation of these receptors simultaneously decreasing β-adrenoreceptor activity via adenylate cyclase inhibition. Additional application of Ca^{2+} ions in our experiments can attenuate the suppressive effects of deep cooling on the immune response, via reducing activity of β-adrenoreceptors and increasing activity of α-adrenoreceptors.

It should be noted that application of Ca^{2+} ions and cold exposure oppositely affect some processes characterizing the development of the immune response. Calcium-dependent thermoregulatory mechanisms developing during deep cooling (thermoreceptor activation, contractile activity of skeletal muscles and vascular smooth muscles) are more prioritized in case when cooling anticipates immunization, and compete for Ca^{2+} ions with processes participating at early stages of the immune response formation. We previously showed [12] that administration of Ca^{2+} ions promotes intensification of systemic cold-protective reactions (restrictory reaction and contractile thermogenesis) and at the same time, according to the results of this work, attenuates the suppressive effects of deep cooling on the immune response.

Our findings indicate the possibility of differential modulation of calcium-dependent mechanisms of the immune response development by exogenous calcium and deep cooling. This suggests that competitive relationships between calcium-dependent processes can serve as a mechanism of the involvement of different system into the formation of the general organism's response to cold exposure.

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