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Temperature-dependent changes in erythrocytes' cytosol state during natural and artificial hypobiosis

S.V. Repina*, O.A. Nardid, V.S. Marchenko, A.V. Shilo

Department of Cryobiophysics, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine, 23, Pereyaslivska str., Kharkiv 61015, Ukraine

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Abstract

At present, the question of how the structural state of the erythrocyte cytosol is arranged to maintain essential permeabilities successfully both at normal temperature and during periods with a significant body temperature reduction during hypobiosis remains unanswered.

In the present work, we performed comparative investigations of temperature-dependent changes in the cytosol state of erythrocytes from animals subjected to natural (winter hibernating ground squirrels) or artificial hypobiosis. The cytosol state was evaluated by the ESR method of spin probes (TEMPON) within the temperature range of 0–50 °C. Erythrocyte resistance to acid hemolysis, which is limited by the permeability of membranes for protons and the state of the anion channel, were determined using the method described by Terskov and Getelson [Biofizika 2 (1957) 259]. A change in cytosol microviscosity of erythrocytes was found as well as a temperature-dependent increase in acid resistance of erythrocytes.

Our investigations allow us to conclude that physiological changes occurring in a mammalian organism during natural and artificial hypobiosis are accompanied by structural modifications of the erythrocyte cytosol.

The temperature range where these modifications are observed (8, 15, 40 °C) suggests that the most probable modifying link is spectrin and/or the sites of its interaction with membrane. The interaction of cytoskeletal components with the cell membrane plays a key role in regulation of membrane permeability, suggesting an important role of this interaction in the adaptive reactions of erythrocytes.
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Keywords: Erythrocyte; Permeability; Cytosol; Adaptation

1. Introduction

Adaptation to environmental conditions is one of the most important and characteristic properties of living systems at any organizational level. In addition to the variety of natural conditions, also ecological consequences of scientific and technical progress and intensive development in medicine, veterinary science and pharmacology necessitate the study of living systems and their reactions to extreme conditions.

The erythrocyte appears to be a good model system for studies of molecular mechanisms of cellular reactions. This cell offers many experimental and interpretative advantages for detection of the role of cellular components and/or their interactions in adaptive or deleterious reactions due to their well-studied structure. The erythrocyte, belonging to the circulatory system, requires an adaptive response not only to allow the organism to exist in extreme conditions, but also to prevent pathological conditions.

Environmental temperature is one of the most important natural factors with regards to adaptation of the organism. The erythrocyte needs to modify its structural and functional components due to temperature dependency of all physical and chemical processes in order to remain functional.

The mammalian hibernators are examples of organisms that have adapted genetically to natural temperature fluctuations. Mammalian hibernation presents a convenient biological and practical system to get insight into the problems of cryogenic banking of human organs and tissues [1].

Available data on cellular mechanisms of natural hypobiosis concerns, mainly, the study of functional features of cellular membranes, including those of RBCs. Characteristics of temperature-dependent passive and active trans-

* Corresponding author. Tel.: +7-380-57-777-2470; fax: +7-380-57-772-0084.
E-mail addresses: srepina@mail.ru, cryo@online.kharkov.ua (S.V. Repina).

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ports of ions and organic molecules and of enzyme systems are well established [2,3]. The structural modifications determining reversal of functions have been investigated insufficiently especially at the cellular level. There is no data explaining how the structural state of the cytosol enables maintenance of metabolic balance in RBCs of hibernators both at 37 and 2–5 °C. Dependency on temperature of all vital biochemical processes proceeding in the RBC cytosol of these organisms necessitates structural changes in response to a change in body temperature.

We have studied models of simulated hypobiosis in non-hibernating mammals to get a better understanding of adaptational cellular mechanisms in response to temperature fluctuations in addition to examination of organisms under natural hypobiosis (winter hibernating ground squirrels).

A hibernator preparing for winter hibernation up-regulates the activity at critical points of homeostatic systems. This phenomenon is modelled most successfully during rhythmic cold exposures of the organism while determining structural and functional changes in the cardiovascular and central nervous systems [4]. Under rhythmic hypothermia, there is a significant rise in resistance to low temperatures.

The hibernative state itself is modelled using a rat, chilled according to the Andjus–Bachmetjev–Giaya model (ABG-model) [5,6], where the rectal temperature was reduced to 16 °C. This was achieved by keeping the rat in a hermetic chamber with ambient temperature of 3–5 °C for 3 h. Thus, this setup modelled hibernation properly: combined hypothermia, hypoxia and hypercapnia.

All vitally important biochemical processes taking place in the cytosol of erythrocytes of hibernating organisms depend on temperature. Thus, structural modification is necessary in response to a body temperature change. A comparative study examining the state of the RBC cytosol, when changing the temperature in vitro within the range 0–50 °C, will reveal structural rearrangements of the components of the cytosol as well as interspecific differences in the adaptive cellular reactions.

2. Materials and methods

Erythrocytes of ground squirrel *Cittelus undulatus* were studied in the states of deep hibernation, arousal and active period during the winter season. Rats were studied after rhythmic hypothermia with a stimulation frequency on the caudal thermoreceptors of 0.1 Hz (cold resistance of anesthetized animals was estimated on basis of the duration of a 0.5 °C change in rectal temperature of animals kept in the cold chamber (4 °C), and on non-anesthetized animals on the duration of active swimming in ice cold water). Furthermore, rats were studied in the state of artificial hypobiosis (ABG-model).

The dynamic structure of the cytosol was investigated by the ESR method of spin probes, using the water-soluble probe TEMPON and the widening agent potassium ferricyanide [7]. The suspension temperature was varied in the "Bruker" ER-100 spectrometer resonator within a range of 0–50 °C.

Acid hemolysis of erythrocytes was carried out as described in Ref. [8]. Changes in light scattering were measured at 700 nm using a Pye Unicam SP 8000 spectrophotometer. In the thermostatted chamber, changes in the temperature at which hemolysis occurred were assessed within the temperature range of 0–50 °C.

3. Results and discussion

Comparative analysis of thermo-induced behaviour of the probe in erythrocytes showed differences both in cytosol microviscosity and in the profile of its temperature dependency, especially below physiological temperatures.

The cytosol microviscosity at 37 °C, characterized by the spectral parameters of the spin probe, was reduced as follows: hibernating ground squirrel ≥ rat after rhythmic hypothermia > active ground squirrel ≥ control rat > aroused ground squirrel ≥ rat according to ABG model. This suggests that structural rearrangements of the erythrocyte cytosol accompanied the adaptive responses to temperature of the whole organism. It is interesting to note that thermal rearrangements, arrested by us in ground squirrel RBC membranes by the lipid probe and reflecting modifications of molecular components, are observed at similar/identical temperatures for erythrocytes of the two mammalians, namely: 8–10, 15, 20, 28–33 and 40 °C [9]. We also found that the temperature dependency of the probe behavior practically coincided for the active state of ground squirrel and the homeothermic mammalian, human, up to a temperature of 15 °C, above which there is a weakening of the spectrin connections to the integral membrane components [10].

The system of thermal rearrangements in the cytosol of active ground squirrel and control rat erythrocytes correlates with those known for membranes, which, however, are connected to modifications of skeletal proteins or membrane lipids (but not of integral protein components), namely: 6–8, 15, 32 and 40 °C (Fig. 1). After rhythmic hypothermia the thermo-induced cytosol behaviour changed: the transitions within the temperature range of 8–40 °C are less marked. However, the areas with abnormal behavior of the probe are not determined, in fact the curve slope changes only at 40 °C. Thus, the reduced slope of the curve is observed after the transition at 8 °C, where modification of a protein 4.1, linking spectrin with the integral membrane components, takes place [11]. Furthermore, the heat stability of spectrin or its connection with the membrane is increased.

The reduction in slope with increasing temperature as well as decreased amount of thermal rearrangements (smoothing) observed throughout the temperature range in

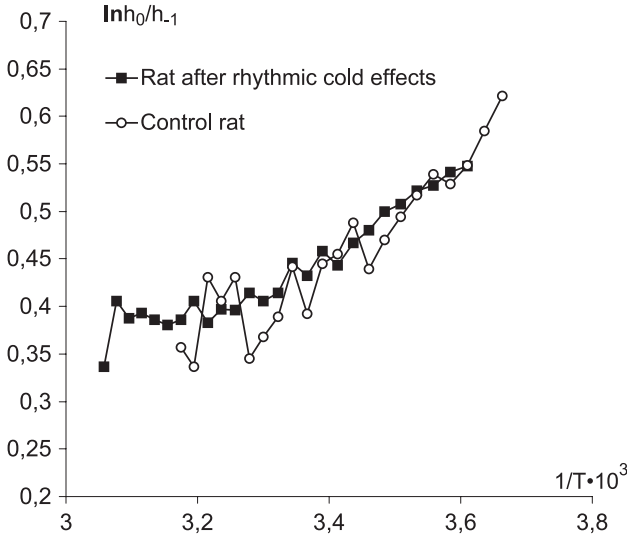

Fig. 1. Arrhenius dependency for the probe mobility in the RBCs of control rat and rat after rhythmic hypothermia.

the Arrhenius plot of erythrocytes from rats after rhythmic hypothermia suggests that the erythrocytes adapt to a change in temperature [2].

In the cytosol of rat erythrocytes treated according to the ABG model, the thermal profile also differed from the profile for erythrocytes from control rat (Fig. 2). The slope of the curve is fairly constant within the temperature range of 8–40 °C. The most highly expressed modifications are associated with skeletal components rearrangements (transition at 8 °C and an irreversible increase in microviscosity, probably due to dehydration of erythrocytes, above 40 °C) and "lipid" transition at 32 °C.

We carried out research of relative RBC resistance to acid hemolysis, which allowed us to estimate the characteristics of the RBC membrane H^+ ion permeability (as an initial stage of acid hemolysis) and of the state of band 3 [12].

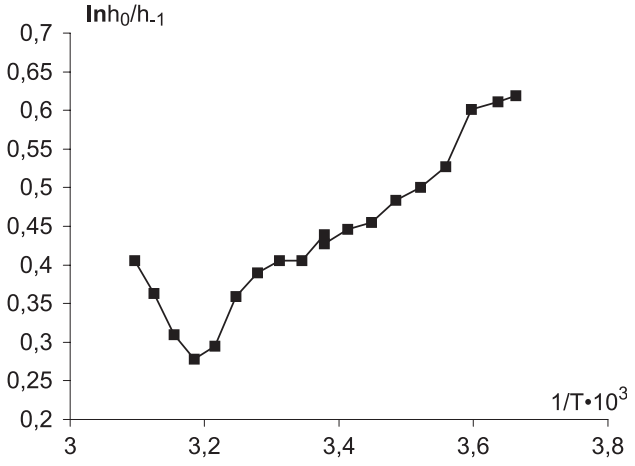

Fig. 2. Arrhenius dependency for the probe mobility in the RBCs of rat according to the Andjus–Bachmetjev–Giaya model.

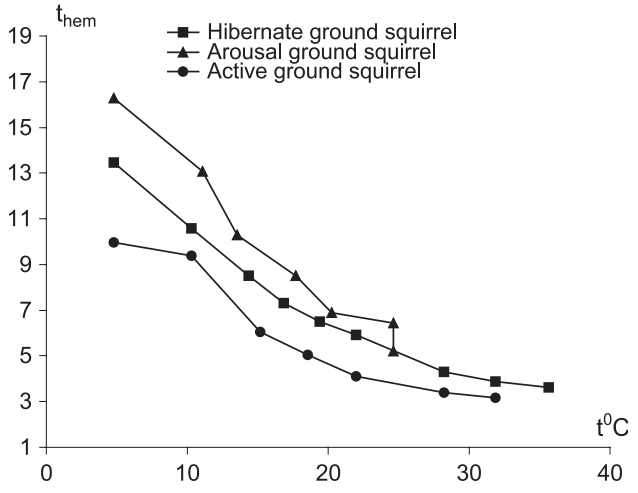

Fig. 3. The temperature dependency of acid hemolysis time of RBCs from hibernating, aroused and active ground squirrels.

Also, it allowed us to examine whether the resistance to acid hemolysis of RBCs varies in erythrocytes exposed to natural and artificial hypobiosis. Taking into account the aim of the research, which was to reveal structural features in connection with adaptive reactions in response to temperature, we used temperature modification of the method.

Therefore, we studied the resistance of the erythrocytes to acid hemolysis in vitro, determined by the duration time until hemolysis was observed at varying temperatures (Figs. 3 and 4). It is clear that throughout the temperature range the erythrocytes of hibernating and aroused animals had an increased resistance to hemolysis compared to erythrocytes from active animals (Fig. 3).

An increased cold-resistance of rats after rhythmic cold exposures, which was registered by an increase in time

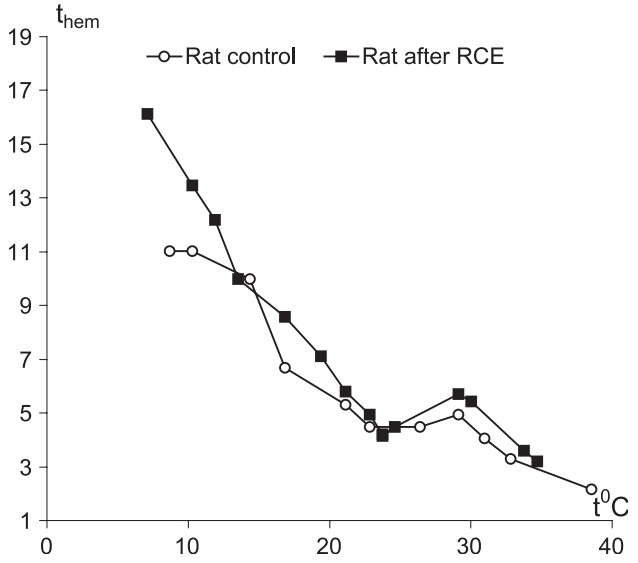

Fig. 4. The temperature dependency of acid hemolysis time of RBCs of control rat and rat after rhythmic hypothermia.

spent in ice water, was accompanied at the erythrocyte level by an increased resistance to acidic hemolysis at temperatures below 20 °C, but most pronounced at temperatures below 8–10 °C. The profiles of the temperature dependencies of acid hemolysis time for one control rat and one rat after rhythmic hypothermia differed only below the temperature corresponding to the temperature at which structural transitions of skeletal components happen (Fig. 4).

4. Conclusions

The data allow us to conclude that the physiological changes taking place in a mammalian organism under natural and artificial hypobiosis are accompanied with structural modifications in the state of erythrocyte cytosol. An increased cold-resistance of all the organisms was accompanied at the level of the erythrocyte by temperature-dependent increase in acid resistance. Of primary interest are the temperatures at which the rearrangements of skeletal components take place. Due to the temperature ranges in which the changes in the state of the cytosol occurred, we suggest that certain molecular cytosol-membrane components are involved in the adaptive cellular response. Despite some variety in the modifications revealed by our analysis, the overall rearrangements appear to involve spectrin and/or its interaction with the membrane. Taking into account the key role of the interaction of cytoskeletal components with the membrane in the regulation of membrane permeability, one can expect an important role of this interaction in the adaptation of erythrocytes to temperature changes.

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