PHYSIOLOGY

Tripeptide Pro-Gly-Pro Prevents Disturbances in Osmotic Resistance of Rat Erythrocytes under Conditions of Inflammation

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The development of inflammation (experimental model of peritonitis induced by administration of sodium thioglycolate) was accompanied by a decrease in osmotic resistance of erythrocytes. Changes in osmotic resistance of erythrocytes associated with preliminary (15 min before induction of inflammation) administration of peptide Pro-Gly-Pro were significantly weaker, and the percentage of hemolyzed cells was reduced. The peptide injected against the background of developed inflammation (1 h 45 min after induction) had no corrective effect on osmotic resistance. During *in vitro* experiments, Pro-Gly-Pro did not affect hemolysis of intact erythrocytes. These results support the assumption that prophylactic administration of the peptide protects erythrocyte membranes and increases their osmotic resistance.

Key Words: *inflammation; peptides; erythrocytes; osmotic resistance*

Changes in blood rheology, namely increased viscosity, aggregation, and impaired osmotic resistance of erythrocytes (ORE), are characteristic of many diseases such as atherosclerosis, diabetes, traumas, surgery, and pathological states (inflammation, stress response) [8,11]. Blood rheology depends on the state of blood cells, primarily erythrocytes. Structural changes in erythrocyte membrane lead to modulation of their functional properties and aggregation, manifesting at the pathophysiological level by microcirculatory disorders resulting in metabolic and functional disturbances in tissues and organs [9]. Evaluation of ORE changes influenced by various exogenous and endogenous factors is important in practical terms [3].

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We have previously demonstrated microcirculation disturbances and impaired contractile activity of rat mesenteric lymphatic vessels during inflammation [7] and different types of stress [4]. Regulatory glyproline peptides are found to prevent or significantly reduce the severity of these disorders [1,5]. We hypothesized that glyprolines can improve ORE under conditions of inflammation.

Here we studied changes in ORE and identified opportunities for its peptide correction during inflammation in rats.

MATERIALS AND METHODS

The study performed on white outbred rats weighing 180-200 g. The rats were housed under standard conditions on a standard laboratory diet with free access to water and food. Peptide Pro-Gly-Pro (PGP) synthe-

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sized at the Institute for Molecular Genetics, Russian Academy of Sciences, was injected intramuscularly in a dose of 3.7 mmol/kg 15 min before induction of inflammation (intraperitoneal administration of 4 g/kg sodium thioglycolate, Fluka [10]). The blood was sampled from the jugular vein and stabilized with 3.8% sodium citrate (9:1). An equal volume of 0.85% NaCl was used as the control.

For evaluation of the structural and functional properties of erythrocyte membranes, osmotic resistance was determined by the method of Idelson [2]. ORE was evaluated by erythrocyte stability in hypotonic NaCl solution. The degree of erythrocyte hemolysis at a certain NaCl concentration was calculated from the ratio of optical density of the solution to that of the probe containing 0.1% NaCl and expressed in percents. Optical density was measured at 540 nm.

The results were processed statistically using Student's *t* test. Experiments were carried out in accordance with international principles of the Declaration of Helsinki on humane treatment of animals.

RESULTS

In series I, the state of erythrocyte membranes was studied in the dynamics of the inflammatory response caused by administration of sodium thioglycolate. Blood samples were collected 30 min, 2 h, and 18 h after induction of inflammation. The degree of hemolysis did not differ from control values 30 min after administration of thioglycolate (Fig. 1, *a*), but after 2 h it decreased and attained significant values at NaCl concentrations of 0.5% and 0.35% ($p<0.001$; Fig. 1, *b*); 18 h after injection of thioglycolate (Fig. 1, *c*) significant differences in the degree of hemolysis were observed only at 0.4% NaCl. These findings suggest that ORE decreased during the development of inflammation. The most significant differences in ORE were observed 2 h after thioglycolate administration.

This is consistent with previous findings that most pronounced impairment of contractile activity of mesenteric lymphatic vessels in rats with experimental peritonitis was observed in 2 h of inflammation [6]. Therefore, this term was chosen for evaluation of the

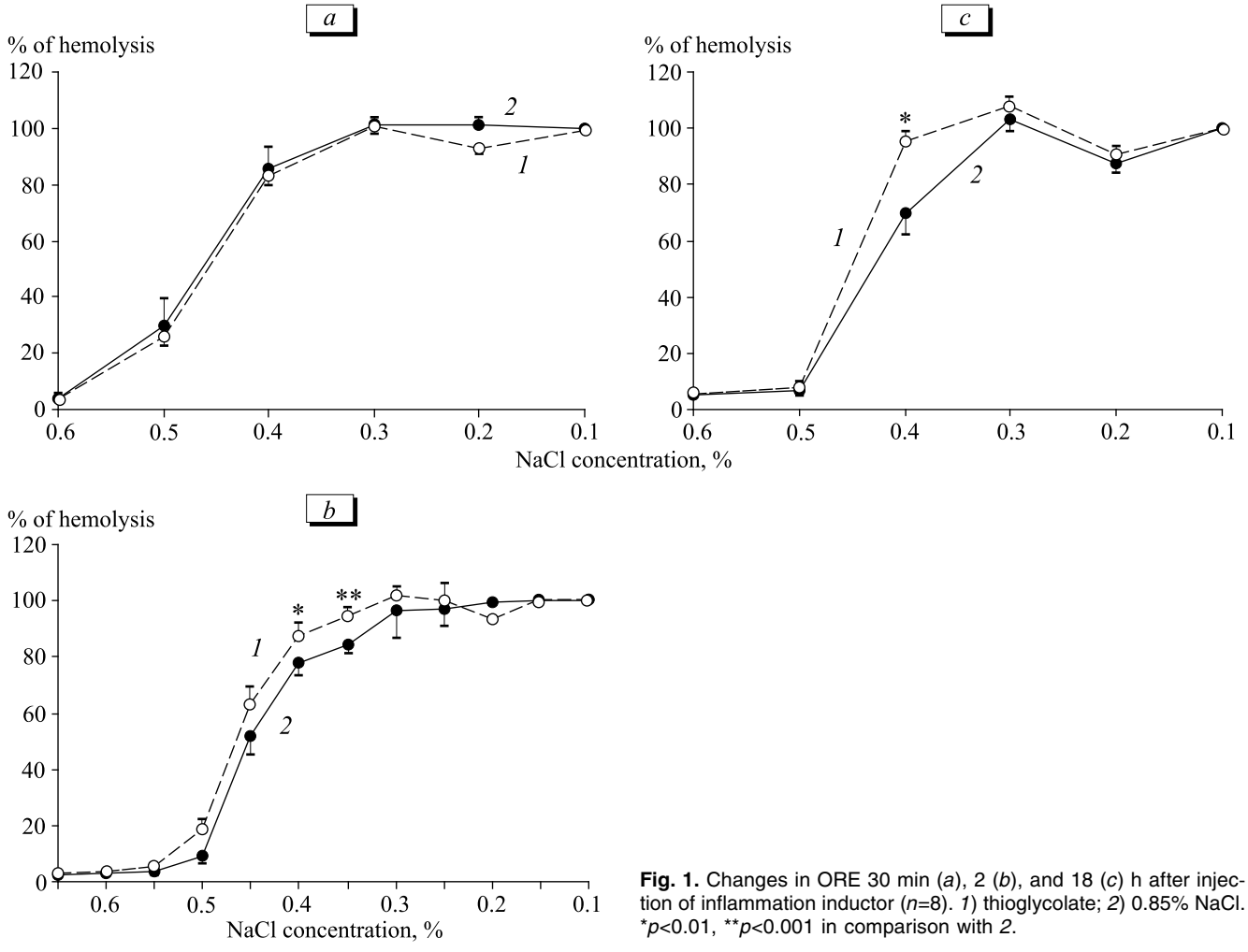

Fig. 1. Changes in ORE 30 min (*a*), 2 (*b*), and 18 (*c*) h after injection of inflammation inductor (n=8). *1*) thioglycolate; *2*) 0.85% NaCl. $^{*}p<0.01$, $^{**}p<0.001$ in comparison with *2*.

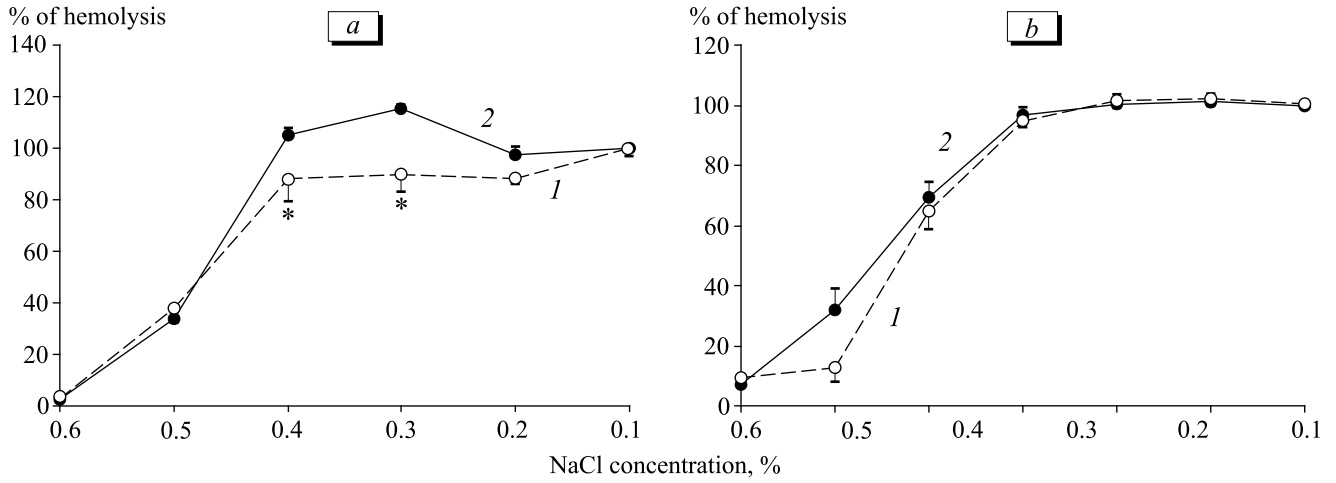

Fig. 2. Changes in ORE after administration of PGP before (*a*) and after (*b*) induction of inflammation (n=8). *1*) PGP; *2*) 0.85% NaCl. *p<0.01 compared with *2*.

effect of peptide PGP on stability of the erythrocyte membranes. In series II, the peptide was administered prophylactically 15 min before injection of thioglycolate.

Changes in osmotic resistance of erythrocytes associated with preliminary (15 min before the induction of inflammation) administration of PGP were significantly weaker and the percent of hemolyzed cells decreased. The differences became significant in solutions containing 0.4 and 0.3% NaCl (Fig. 2, *a*). The peptide injected against the background of established inflammation (1 h 45 min after induction of inflammation) had no corrective effect on osmotic resistance (Fig. 2, *b*). *In vitro* experiments have also demonstrated that PGP did not affect hemolysis of intact erythrocytes.

These results support the assumption that the peptide administered in prophylactic mode protects erythrocyte membranes and increases their osmotic resistance.

Changes in physicochemical properties of erythrocyte membranes are known to be the markers of inflammation and evaluation of these chances by measuring cell resistance to various influences is important so that we might better understand and manage the mechanisms underlying inflammation.

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