Role of Purinergic Receptors of Erythrocytes in the Regulation of Conformation and Oxygen- and NO-Transporting Capacity of Hemoglobin

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Raman spectroscopy was used to investigate changes in hemoglobin conformation and its ability to transfer O₂ and NO induced by activation of purinergic receptors of erythrocytes with extracellular ATP. It was found that addition of ATP in a final concentration of 0.1 mM and higher to erythrocyte suspension was followed by changes in hematoporphyrin conformation, while addition of ATP in a concentration of 1 mM and higher increased the proportion of oxyhemoglobin and NO-associated hemoglobin complexes. In case of purinergic receptors activation in blood erythrocytes, significant changes in hemoglobin conformation were observed only at a final ATP concentration of 5 mM, probably due to buffer properties of the blood.

**Key Words:** ATP; hemoglobin; erythrocytes; Raman spectroscopy; purinergic receptors

ATP is known not only provides cell metabolism, but also performs signaling function, being the main ligand of purinergic receptors (PR) [7]. ATP content strongly varies depending on the cell type. Tumor cells and epithelial cells of the eye lens are characterized by high levels of intracellular ATP. In neurons, typical ATP concentrations are 2-5 mM in the cytoplasm and higher (up to 100 mM) in synaptic vesicles [3]. It was formerly thought that the appearance of ATP in the extracellular space is a result of its release from damaged or dead cells. At present, the elevated extracellular ATP concentration is believed to result from its release from healthy cells, which is of high physiological significance. ATP is released by neurons of the central and peripheral nervous systems. Moreover, many other cell types can secrete ATP into the extracellular space under the effect of mechanical deformation, osmotic swelling, or chemical agents.

Plasma level of ATP is maintained due to activity of phosphatases, ectoATPases, and ectonucleases [11]. Among PR, two receptor families (P2X and P2Y) are distinguished differing by their structure and signal transduction mechanisms [6,10]. It is known that extracellular ATP and products of its hydrolysis interact with PR on blood cells, including erythrocytes [7]. However, the role of erythrocyte PR activation in modulation of O₂-transporting and NO-binding capacities of erythrocyte hemoglobin remained almost unexplored.

The aim of this study was to investigate the role of erythrocytic PR activation in the shift in hematoporphyrin conformation and its ability to carry O₂ and NO.

**MATERIALS AND METHODS**

The study was carried out on whole blood erythrocytes of healthy people and red blood cells, washed by three-stage centrifugation (5 min, 3000 rpm) in Alen saline (in mM): 145 NaCl, 5 KCl, 1.0 CaCl₂,
1.0 MgSO$_4$, 4 Na$_2$HPO$_4$$\times$12H$_2$O, 1.0 NaH$_2$PO$_4$$\times$2H$_2$O, 10 C$_6$H$_{12}$O$_6$ (pH 7.4).

Conformation of hematoporphyrin, the prosthetic group of hemoglobin, and the proportion of hemoglobin complexes with O$_2$ and NO were evaluated by Raman spectroscopy ($\lambda$=473 nm, power 18-20 mW). Changes in the ratio of intensities (I) of specified Raman spectrum bands reflect changes in hematoporphyrin conformation [4], which can be interpreted as the contribution of oxyhemoglobin complexes to the total amount of hemoglobin complexes (I$_{1375}$/(I$_{1375}$+I$_{1355}$) O$_2$-binding capacity of hemoglobin (I$_{1355}$/I$_{1375}$), O$_2$-releasing capacity of hemoglobin (I$_{1375}$/I$_{1552}$), and the proportion of NO–hemoglobin complexes (I$_{1618}$/I$_{1355}$+I$_{1375}$) [1,2,4].

Initially, blood and erythrocyte samples showed different level of hemoglobin oxygenation. To eliminate this effect, the parameters measured by Raman spectroscopy after addition of ATP were standardized to the same parameters measured in the absence of ATP.

Fig. 2. Effect of extracellular ATP on the kinetics of proportion of oxyhemoglobin (a, b) and NO-hemoglobin (c, d) complexes in erythrocytes (a, c) and whole blood (b, d). Here and in Fig. 3: the data are presented as a ratio of the parameter measured at a certain time point after ATP addition to its initial value. Significant differences in the parameters under study ($p<0.05$) for erythrocyte suspension caused by ATP in final concentrations of 0.1 (**), 0.5 (**), 1 (***), and 5 (****) mM and in the whole blood after ATP addition in final concentrations of 5 (++) mM.

Fig. 1. Typical Raman spectra of hemoglobin of erythrocytes in the incubation medium (1) and in whole blood (2). Dashed line shows peaks used for assessment of the analyzed parameters.