# Band 3 protein-mediated chloride gradient is the driving force for ammonia/ammonium influx in human red blood cells

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**J. Sudnitsyna**[I,II], S. Gambaryan[II], T. Ruzhnikova[II,III], I. Mindukshev[II]

[I]Center for Theoretical Problems of Physico-Chemical Pharmacology, RAS, Moscow, Russia, [II]Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, Saint-Petersburg, Russia, [III]Saint Petersburg State Institute of Technology (Technical University), Department of Molecular Biotechnology, Saint-Petersburg, Russia

Ammonium/ammonia (AM) Rhesus transporters are expressed in tissues involved in AM generation, secretion, and excretion, however, the physiological role of erythroid RhAG glycoprotein is still unclear. AM concentration in red blood cells (RBCs) is approximately three times higher than in plasma, and RBCs are the only blood cells that swell and lyse in isotonic $NH_4^+$ media, indicating that AM is transported inside the cell. However, the real driving force for AM influx in RBCs is still not clear.

RhAG and anion exchanger 1 (band 3, AE1) form a structural complex in erythroid membrane, therefore we hypothesized that AE1 might be involved in AM transport in RBCs.

Washed human RBCs were resuspended in HEPES buffer (NaCl, 140мM) and analyzed by flow cytometry and laser diffraction. To evaluate the AE1 role in AM transport, we (i) inhibited AE1 by DIDS in $NH_4^+$ media ($NH_4Cl$, 140 mM), (ii) added $HCO_3^-$ (0-25mM) equimolarly to isotonic $NH_4^+$ media, (iii) dose-dependently substituted $Cl^-$ by glutamate in $NH_4^+$ media until complete disposal of the $Cl^-$ gradient.

In isotonic $NH_4^+$ media the AE1 substrate $HCO_3^-$ dose-dependently increased hemolysis rate, and inhibition of AE1 completely blocked hemolysis. In isotonic $NH_4^+$ media where $Cl^-$ was substituted by glutamate altering of the $Cl^-$ gradient dose-dependently decreased hemolysis rate until the complete prevention of cell swelling and hemolysis in absence of $Cl^-$ gradient.

Our data strongly indicate that erythroid RhAG and AE1 are functionally connected, and AE1-mediated $Cl^-$ gradient drives AM transport against its gradient via RhAG. Taken together our data revealed the new mechanism of AM transport against the gradient in RBCs and suggested that RBCs in addition to main functions are involved in regulation of AM concentration in plasma. This study was supported by the RFBR, grant No. 19-315-60015 to JS.