Rate of Activation and Deactivation of K:Cl Cotransport by Changes in Cell Volume in Hemoglobin SS, CC and AA Red Cells

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Abstract. Red blood cells (RBC) of subjects homozygous for hemoglobin A (AA), C (CC) and S (SS) exhibit different cell volumes which might be related to differences in cell volume regulation. We have investigated how rapidly K:Cl cotransport is activated and deactivated to regulate the cell volume in these cells. We measured the time course of net K⁺ efflux after step changes in cell volume and determined two delay times: one for activation by cell swelling and a second for deactivation by cell shrinkage. Cell swelling induced by 220 mOsm media activated K⁺ efflux to high values (10–20 nmol/liter cell x hr) in CC and SS; normal AA had a threefold lower activity. The delay time for activation was very short in blood with a high percentage of reticulocytes (retics): (SS, 10% retics, 1.7 ± 0.3 min delay, n = 8; AA, 10% retics, 4 ± 1.5 min, n = 3; CC, 11.6% retics, 4 ± 0.3, n = 3) and long in cells with a smaller percentage of reticulocytes: (AA, 1.5% retics, 10 ± 1.4 min, n = 8; CC whole blood 6% retics, 10 ± 2.0 min, n = 10, P < 0.02 vs. SS). The delay times for deactivation by cell shrinking were very short in SS (3.6 ± 0.4 min, n = 8, P < 0.02) and AA cells with high retics (2.7 ± 1 min, n = 3) and normal retics (2.8 ± 1 min, n = 3), but 8–15-fold longer in CC cells (29 ± 2.8 min, n = 9).

Density fractionation of CC cells (n = 3) resulted in coenrichment of the top fraction in reticulocytes and in swelling-activated cotransport (fourfold) with short delay time for activation (4 ± 0.3 min) and long delay for deactivation (14 ± 4 min). The delay time for activation, but not for deactivation, increased markedly with increasing cell density. These findings indicate that all CC cells do not promptly shut off cotransport with cell shrinkage and high rates of cellular K⁺ loss persist after return to isotonic conditions.

In summary, (i) K:Cl cotransport is not only very active in young cells but it is also very rapidly activated and deactivated in young AA and SS cells by changes in cell volume. (ii) Delay times for cotransport activation markedly increased with RBC age and in mature cells with low cotransport rates, long delay times for activation were observed. (iii) The long delay time for deactivation exhibited even by young CC cells induces a persistent loss of K⁺ after cell shrinkage which may contribute in vivo to the uniformly low cell volume, low K⁺ and water content of CC cells.

Key words: Hemoglobin S — Hemoglobin C — Red blood cells — KCl cotransport — Sickle cell anemia — Cell volume regulation

Introduction

Upon maturation of human and some mammalian reticulocytes, cell volume, K⁺ and water content decrease markedly and mean corpuscular hemoglobin concentration (MCHC) increases with cell age [31, 40]. An important characteristic of young human red blood cells (RBC) is their elevated activity of volume-stimulated K:Cl cotransport, a powerful regulator of cell volume that responds to cell swelling or reduced pH by activating a net loss of K⁺, Cl⁻, and water to re-establish normal cell volume [8, 9, 13, 16, 23, 25]. The physiological
stimulus for activation of K:Cl cotransport may be the reduced pH characteristic of certain organs such as the kidney or conditions such as low pO2 or stress.

Several studies independently demonstrated that in RBCs of subjects homozygous for hemoglobin A (AA) and homozygous for hemoglobin S (SS) [9, 13], the high cotransport activity of young red cells decreases with cell age and increased MCHC [for reviews, see 6, 12, 29]. In mature RBCs of AA subjects, cotransport activity is very low but it has been extensively characterized for its anion dependence, effect of inhibitors and K+ kinetic properties [12, 20, 33]. In SS and CC patients, swelling-stimulated K+ efflux is very active [7, 8] because the red cell life span is shortened to approximately 10–14 and 40 days, respectively [34]. As expected from the differences in hemolytic intensity between the two entities, CC whole blood has a mildly elevated reticulocyte count (average 7%) which is less than that of SS whole blood (average 16%) [22, 46]. In spite of the smaller reticulocyte count in CC blood, swelling-stimulated cotransport is very active and nearly equal to that of SS cells [3, 8, 16]. CC cells have a uniformly higher MCHC, a smaller mean corpuscular volume (MCV), lower cell water content and higher density than SS or AA cells because of their low K+ content [22, 36, 46]. In contrast, SS cells do not exhibit a uniform increase in MCHC and the majority of young red cells in SS blood have a normal MCHC and MCV although there is a variable population of dense red cells [7, 12, 13, 16, 23]. In SS cells, generation of dense and irreversibly sickled cells (ISCs) is strongly associated with enhanced Ca2+ influx, elevation of cytosolic Ca2+ levels and opening of the Ca-activated K+ channels which are inhibited by charybotoxin [for reviews, see 6, 12, 29]. Thus, even though cotransport is very high in CC cells and its activation can lead to volume reduction after cell swelling [8], this property is not sufficient to account for the homogenous elevation of MCHC of CC whole blood. The mechanism of the uniformly increased MCHC of CC cells has remained unresolved.

For a physiologically effective regulation of cell volume, cell swelling must rapidly signal the activation of a net efflux of K+ and Cl− to reduce MCHC; and, likewise, cell shrinking must signal a rapid deactivation after the initial cell volume is re-established. The hypothesis that we propose to account for the higher density and uniformly reduced volume of CC cells is that changes in cell volume abnormally regulate the K:Cl cotransporter. One mechanism that can contribute to K+ loss and dehydration is an extended delay in turning off cotransport once the volume has returned to normal values. As a first step to define the role of cotransport regulation in determining the final red cell volume, we have investigated how K:Cl cotransport responds to step changes in cell volume of SS, CC and AA cells. To this end, we have followed the time course of K+ efflux from these three cell types to determine the delay times for activation by cell swelling and deactivation by cell shrinking. We found that in young AA, SS and CC cells cotransport has a short delay for activation which appeared to increase with cell age. Cotransport deactivation by cell shrinkage was independent of cell age and had a very short delay time in SS and in AA cells. In contrast, in CC cells cotransport responds very slowly to a reduction in cell volume, an abnormality which can account for an enhanced cell K+ loss and may contribute to their dehydration.

Materials and Methods

Patient Material

Blood was drawn from six SS and four CC patients followed in the Heredity Clinic of the Bronx Comprehensive Sickle Cell Center after informed consent. We also studied six normal AA subjects with normal reticulocyte counts and three with high reticulocyte counts due to nutritional anemia. The hemoglobin of all patients was characterized by a combination of cellulose acetate (pH 8.6) and citrate agar (pH 6.4) electrophoresis and a solubility test for hemoglobin S [17].

Measurements of Reticulocytes

Reticulocytes were determined by suspending aliquots of cells in centrifuged plasma at hematocrit 50%. Equal volumes of blood and new methylene blue reticulocyte stain were mixed and the samples were allowed to incubate at room temperature for at least 10 min after which smears were made for counting.

Measurements of K:Cl Cotransport Activity

RBC Preparations

RBCs used in these experiments were washed four times with cold preservation solution containing (mm): 135 KCl, 15 NaCl, 10 Tris-MOPS, pH 7.4 at 4°C and shipped overnight on ice to Boston [16]. Transport experiments were performed the next day in Boston, within 24 hr after the blood was drawn. RBCs were washed with cold washing solution (CWS) containing (mm): glucose-NaO3 149, MgNO3 1, Tris MOPS 10, pH 7.4, 4°C. A 50% cell suspension was made in CWS to measure hematocrit, hemoglobin, K+ (500 dilution), and Na+ (50 dilution) as previously described [13, 23]. Control experiments to study the effect of 24 hr preservation were done at the Albert Einstein College of Medicine, Bronx, New York.

K:Cl Cotransport Activation by Cell Swelling

We have chosen to use measurements of net K+ efflux into Na+ media instead of Rb+ influx to study the time course of cotransport activation and deactivation. Previous studies in rabbit and LK sheep RBCs [18, 27] have measured Rb+ influx to avoid interference by cell lysis. As previously reported by us and others [7, 8, 13], SS and CC cells have a very high activity of K:Cl cotransport that is 3-10 times higher than that of rabbit and LK sheep cells which minimizes errors of net K+ efflux measurements. Net K+ efflux was determined as previously described [13, 16] incubating cells in K-free (reagent contamination was determined to be 5 μM) Na+ media of varying osmolality, pH and Cl−.