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PARTIAL RESTORATION OF SODIUM AND POTASSIUM GRADIENTS BY HUMAN ERYTHROCYTE MEMBRANES*

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Summary

Sodium-loaded human erythrocyte ghosts, incubated for 24 h in medium containing low external potassium and high external sodium, catalyzed net movements of sodium and potassium against their respective concentration gradients, resulting in partial restoration of cation gradients.

Intact human erythrocytes are capable of net transport of sodium and potassium ions against concentration gradients [1,2]. Extensive investigations, primarily utilizing isotope tracer kinetics, have led to the widely accepted view that a pumping mechanism for selective cation transport resides in the plasma membrane, and that energy is derived continuously from a membrane-localized ATPase which utilizes internal ATP and is asymmetrically and selectively stimulated by internal sodium and external potassium and inhibited by external ouabain (reviewed in refs. 3 and 4). Substantial evidence for the identification of the $(Na^+ + K^+)$-ATPase with a component of the sodium pump resulted from studies with isolated human erythrocyte membranes, commonly called "resealed ghosts", which retain sidedness and the ability to catalyze active transport of sodium and potassium [5—12].

An early report [5] on ghost net potassium accumulation was questioned [11,13,14] on the basis that a high cell concentration during lysis possibly resulted in contamination of ghosts by intact cells, a defect which was corrected in later preparations of ghosts (see ref. 15 for review). Prior to a recent report by Bodemann and Hoffman [12] of ouabain-sensitive potassium accumulation of 1.9 mM and sodium extrusion of 2.9 mM during 3-h incubations, no other direct measurements of net sodium movements

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against a gradient were found in the red cell ghost literature (see ref. 16 for review). This report extends the characterization of resealed ghosts by showing that net movements of sodium and potassium against concentration gradients can be sustained for at least 24 h of in vitro incubation, resulting in partial restoration of cation gradients.

Preparation of resealed ghosts was based on the methods of Hoffman [9, 11], Hoffman et al. [17], Garrahan and Glynn [18,19], and Passow and collaborators [13,20,21]. Fresh human blood from a healthy donor was collected by venepuncture into heparinized vacutainers and washed three times in the following solution: NaCl (140 mM), KCl (10 mM), $MgCl_2$ (2 mM), Tris·HCl (10 mM), Na_2 EDTA (0.1 mM), pH 7.7 at 25°C. The washed packed red blood cells (20 ml) were then rapidly syringed into a fleaker containing 200 ml ice-cold, well-stirred lysis solution of the following composition: Na_2 ATP (5 or 6 mM), $MgCl_2$ (7 or 12 mM), L-cysteine (1 mM), Tris·HCl (10 mM), Na_2 EDTA (0.1 mM), adjusted with 1 M NaOH to pH 6.0 at 3°C. According to Lepke and Passow [21], hemolysis at pH 6 in the cold gives the maximum yield of resealed ghosts. The hemolytic ratio of 10% implies that the hemoglobin concentration in the ghosts is reduced by about an order of magnitude [9].

After 10 min in the lysis solution at 1°C, a resealing solution (20 ml) of the following composition was added to restore normal tonicity: NaCl (0.5 M), KCl (15 mM), sucrose (2 M). The pH was then adjusted to 8.4 at 1°C with 1 M NaOH, and the suspension was incubated at 37°C for 50 min. The sodium-loaded ghosts were then washed four times by centrifugation (15 000 rev./min for 15 min) and resuspension in the following medium: NaCl (50 mM), $MgCl_2$ (2 mM), Tris·HCl (10 mM), Na_2 EDTA (0.1 mM), sucrose (200 mM), pH 8.3 at 5°C. A yield of 12 ml ghosts from the original 20 ml packed erythrocytes was obtained at this step.

A ghost portion (5 ml), resuspended in an equal volume of ghost wash solution, was then layered onto a sucrose cushion (35 ml) consisting of 43% sucrose in Tris·HCl (10 mM) and NaCl (25 mM), pH 8.3 at 5°C, and spun for 90 min at 17 000 rev./min. Bodemann and Passow [13] showed that the leaky ghosts sediment, leaving the resealed ghosts floating as a pink upper band. The ghosts (2 ml) were pipetted carefully off the top of the sucrose cushion, and washed once in the ghost washing solution, and then centrifuged. To the washed ghosts were added 8 ml of cold ghost incubation solution of the following composition: NaCl (50 mM), KCl (10 mM), $MgCl_2$ (2 mM), Tris·HCl (10 mM), Na_2 EDTA (0.1 mM), inosine (10 mM), adenine (10 mM), sucrose (160 mM), pH 7.4 at 37°C. The ghost preparation took about 6 h, and had an overall yield of about 10% by this technique.

The ghosts were incubated at 37°C at 20% hematocrit. At desired time intervals, samples (0.4 ml) were spun in microcentrifuge tubes (A.H. Thomas). The ghost pellets were extracted in HCl for flame photometric analysis of potassium and sodium contents, or analyzed gravimetrically for water content (see ref. 22).

A net increase of potassium and net decrease of sodium contents were observed in the ghosts when incubated in the presence of low external potassium concentration and high external sodium concentration (Table I and Fig. 1).

TABLE I

NET ACCUMULATION OF POTASSIUM AND EXTRUSION OF SODIUM AGAINST CONCENTRATION GRADIENTS BY HUMAN ERYTHROCYTE MEMBRANES

Numbers indicate ion concentrations (mM) or contents (μmol/g ghosts).

	Expt. A	Expt. B
Duration of incubation	24 h	20 h
Initial ATP concentration	5	6
External ion concentration		
Potassium	10	10
Sodium	50	50
Initial internal ion content		
Potassium	10	13
Sodium	60	48
Final internal ion content		
Potassium	22	25
Sodium	35	26
Net change in internal ion content against concentration gradient		
Potassium	+12	+12
Sodium	—15	—22

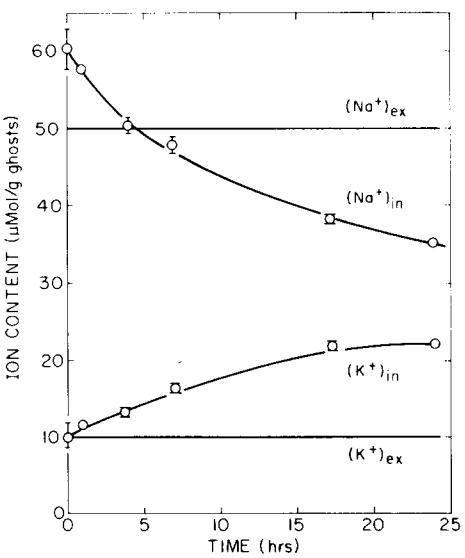

Fig.1. Net accumulation of potassium and extrusion of sodium against concentration gradients by human erythrocyte ghosts. Points with bars represent mean and standard deviation for triplicate analyses of ion content.

In three other experiments with external sodium concentration ranging up to 150 mM, similar net movements of sodium and potassium against concentration gradients also occurred (see ref. 22). The net changes in ion contents against the apparent gradients were computed as the differences between the final ion contents in the ghosts and the constant external ion concentrations (Table I). Since the water content was constant at 95%, the conclusions do not depend on whether the ion contents are expressed on a wet-weight or water basis.

The rates of net potassium accumulation and sodium extrusion shown in Fig. 1 agree with the initial rates determined by Bodemann and Hoffman [12]

in 3-h incubations, but the extent of net movements of both potassium and sodium were considerably larger due to the longer incubation time. The concentration distribution ratio reached for sodium was 0.7 and for potassium was 2.3. These gradients are much lower than the normal sodium ratio of 0.1 and potassium ratio of 36 [23]. Limiting factors for reconstituting normal cation gradients by isolated membranes include the energy supply, the efficiency of pumping, the duration of incubation, and the passive permeability of the membrane. Selective potassium accumulation and sodium extrusion against concentration gradients by resealed ghosts during sustained incubation is in marked contrast to the failure of leaky membranes [24] and hemoglobin solutions [25—27] to exhibit this basic cellular property.

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