

Properties of the Na⁺-K⁺ Pump in Human Red Cells with Increased Number of Pump Sites

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Abstract

We studied the Na⁺/K⁺ pump in red cells from an obese human subject (MAJ) in which the number of pumps/cell was 10–20 times higher than normal. Through measurements of the kinetic properties of several modes of operation of the Na⁺/K⁺ pump we determined that the pumps in MAJ cells are kinetically normal. In the presence of adequate metabolic substrate the maximum rates of Na⁺ pumping and lactate production saturated at 60 and 12 mmol/1 cell per h, respectively. Under physiological conditions pump and “leak” Na⁺ fluxes were similar in MAJ and normal cells. Since internal Na⁺ was lower in MAJ than in normal cells (Na_i⁺ ~ 2 and 8 mmol/1 cell, respectively), we conclude that the reduction in cell Na⁺ allows the Na⁺/K⁺ pump in MAJ cells to operate at lower fraction of maximum capacity and to compensate for the increased number of pumps.

Introduction

It has been reported previously by DeLuise and Flier (1) that the red cells of a morbidly obese patient (MAJ) contain between 10 and 20 times more Na⁺/K⁺ pumps per cell than is the case with red cells from normal human subjects. This paper reports the results of experiments designed to answer two sets of questions. First, are the kinetic properties of the Na⁺/K⁺ pump in MAJ cells normal? Second, what are the physiological consequences of the increased number of Na⁺/K⁺ pumps in MAJ cells?

To answer the first group of questions, we measured the maximum transport rate and the concentrations of internal and external Na⁺ and/or K⁺ required to activate half-maximally ($K_{0.5}^a$) several modes of operation of the Na⁺/K⁺ pumps in MAJ red cells; exchange of Na_i⁺-K_o⁺ (normal mode), K_i⁺-Na_o⁺ (reversal), Na_i⁺-Na_o⁺, K_i⁺-K_o⁺ and uncoupled Na⁺ efflux (2). In all of these modes, the measured kinetic properties of the Na⁺/K⁺ pumps in MAJ red cells were the same as those observed in red cells from normal human subjects. Therefore, the lesion in MAJ cells seems to involve the number, not the kind of Na⁺/K⁺ pumps.

To answer the second set of questions, we measured the cellular concentrations of Na⁺, K⁺, and ATP, the ouabain-sensitive and ouabain-insensitive fluxes of Na⁺ and K⁺, and the rate of lactate production in MAJ cells incubated in vitro in a medium similar to normal blood plasma. The magnitudes of

the ouabain-sensitive and ouabain-insensitive Na⁺ and K⁺ fluxes and the rate of glycolysis in MAJ cells were found to be similar to those observed in normal control cells incubated under these conditions. However, the concentration of Na⁺ in MAJ cells was 2.2 mmol/1 cell, substantially less than the value observed in normal control cells (8.0 mmol/1 cell). When the concentration of Na_i⁺ in MAJ cells was increased to the value observed in normal cells, the ouabain-sensitive Na⁺ and K⁺ fluxes and the rate of lactate production in MAJ cells were 10–20 times greater than normal provided that MAJ cells were supplied with adequate metabolic substrate. Therefore, MAJ cells compensate for their high pump density by reducing Na_i⁺ to a level that produces ouabain-sensitive fluxes of Na⁺ and K⁺ that are normal expressed on a per cell or per cell volume basis. Under these conditions, the uphill fluxes of Na⁺ and K⁺ through the pump are equal and opposite to the downhill movements of Na⁺ and K⁺ through several pathways; e.g., Na⁺/K⁺/Cl⁻ cotransport, NaCO₃⁻/Cl⁻ exchange, and electrodiffusion (3). The magnitudes of these non-pump pathways appear to be normal in MAJ cells. Thus, the reduction in Na_i⁺ seems to allow MAJ cells to function normally with respect to cation transport and regulation of cell volume.

Methods

Preparation of red blood cells

Blood was collected in heparinized Vacutainer tubes and centrifuged in a Sorvall centrifuge (RB 5B, DuPont instruments, Sorvall Biomedical Div., Newtown, CT) at 5°C for 10 min at 3,000 g. Plasma and buffy coat were carefully removed and the cells washed four times with a washing solution containing 150 mM choline chloride, 1 mM MgCl₂, 10 mM Tris-4-morpholinopropanesulfonic acid (MOPS),¹ pH 7.4 at 4°C (CWS). An aliquot of cells was then suspended in an approximately equal volume of CWS, and from this original cell suspension hematocrit (Hct), cell Na⁺ (1:30 dilution for low-Na⁺ cells and 1:50 dilution for normal Na⁺ cells in 0.02% Acationox), cell K⁺ (1:500 dilution) and hemoglobin (optical density at 540 nm) were determined. The cell Na⁺ and K⁺ content was determined by atomic absorption spectrometry (model 5000, Perkin Elmer Corp., Norwalk, CT).

Change of the intracellular cation composition

In some experiments we altered the intracellular cation content by the nystatin procedure (4). However, preliminary experiments indicated that MAJ cells are depleted of ATP when their internal Na⁺ is increased with the nystatin procedure, which requires washes at 37°C to remove the ionophore. To avoid ATP depletion, Na⁺ loading in MAJ cells was carried out with Na-salicylate, which has been found to increase reversibly the cell permeability to cations, with maximal effect of 0°C (5).

Salicylate loading procedure. 4 ml of washed packed red cells were incubated for 1.5 h in 20 ml of salicylate loading solution at 0°C. The loading solution contained 10 mM Tris-MOPS, pH 7.4 at 0°C, 1 mM

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1. Abbreviations used in this paper: CWS, choline washing solution; Hct, hematocrit; HPLC, high performance liquid chromatography; IAP, inosine, adenine, and inorganic phosphate; MOPS, 4-morpholinopropanesulfonic acid; TEA, tetraethylammonium.

MgCl₂ and 140 mM Na⁺ with different ratios of Na-salicylate and NaCl according to the desired cell Na⁺. Experiments with control cells showed that each 10 mM of Na-salicylate present in the loading solution yields a 1.5 mmol/l cell increase in the intracellular Na⁺ content after 1.5 h incubation at 0°C. With this loading procedure the increase in cell Na⁺ is balanced by a similar loss of K⁺ and the total cation content of the cells is constant. After loading, salicylate and extracellular cations were removed by washing the cells five times at 4°C with choline washing solution. An aliquot of cells was set aside to measure cell Na⁺, K⁺, and hemoglobin. The recovery of the original cell cation permeability was assessed by measuring the variation in cell Na⁺ and volume produced by a 2-h incubation of the cells in isotonic NaCl solution at 4°C. We also determined the rate constant of the ouabain-resistant Na⁺ efflux (at 37°C) in cells depleted of Na⁺ and then reloaded with salicylate to their original cation content. No differences were found between salicylate treated and fresh cells. The ouabain-sensitive Na⁺ efflux, lactate production and ATP/ADP content of control, salicylate- and nystatin-loaded cells were also similar.

Measurement of cation fluxes

Unidirectional radioactive Na⁺ and K⁺ fluxes. We measured the Na⁺ and K⁺ unidirectional fluxes using ²²Na⁺ (Amersham Corp., North Chicago, IL, sp act 1 Ci/mg) and ⁸⁶Rb⁺ (Amersham Corp., sp act 5 mCi/mg) as tracers. The protocol for unidirectional radioactive cation fluxes was similar to that previously described (4), except that in order to meet conditions of linear initial rate, shorter times (2 and 7 min) were used in MAJ experiments and that the loading of fresh MAJ cells with the isotopes was carried out by incubating 6 ml of packed cells for 3 h at 37°C in 6 ml of a solution containing 140 mM NaCl, 4 mM KCl, 10 mM glucose, 2.5 mM K-phosphate buffer, and either 30 μCi/ml of ²²Na⁺ or 50 μCi/ml of ⁸⁶Rb⁺. The fluxes were measured in Na⁺ medium (140–130 mM NaCl, 0–10 mM KCl, 1 mM MgCl₂, 10 mM glucose, 10 mM Tris-MOPS) and in Na⁺-free medium where NaCl was replaced by either choline chloride or tetraethylammonium (TEA). TEA was chosen as an alternative Na⁺ replacement because it mimics the effect of external Na⁺ as a competitive inhibitor of active K⁺ influx but eliminates the Na_i⁺-Na_o⁺ exchange component of the ouabain-sensitive Na⁺ efflux.

Measurement of ouabain-sensitive Na⁺ efflux by atomic absorption spectrometry

We determined the net ouabain-sensitive Na⁺ efflux from MAJ fresh and Na⁺-loaded cells into choline chloride medium at different external K⁺ concentrations (K_o⁺). The medium contained 140–130 mM choline chloride, 1 mM MgCl₂, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4 at 37°C, and different K⁺ concentrations from 0 to 10 mM, in the presence and absence of ouabain (0.1 mM). To avoid ATP depletion during the time course of the fluxes the flux media contained inosine (1.7 mM), adenine (3.3 mM), and K-phosphate buffer, pH 7.4 (2.4 mM) (6). The cells were added to the prewarmed media (~ 19 ml), to provide a final Hct of 1–2%. At different time intervals, three samples of 1 ml cell suspension were delivered to 1.5-ml Eppendorf tubes containing 0.3 ml dibutyl phthalate (Fisher Scientific Co., Fair Lawn, NJ) and immediately spun for 10 s at 13,000 rpm in an Eppendorf microcentrifuge. The supernatant, separated from the cells by the phthalate layer, was removed and its Na⁺ concentration was measured by atomic absorption spectrometry. Fluxes in the presence of ouabain were measured at higher Hct (4–5%) and longer incubation times. The Na⁺ efflux in millimoles per 1 cell per hour was determined as previously reported (4).

Dose-response effect of ouabain on the ⁸⁶Rb⁺ uptake by MAJ cells

We determined the inhibition of the ⁸⁶Rb⁺ influx by increasing concentrations of ouabain (from 10⁻⁹ to 10⁻³ M) in MAJ and in control fresh and low-Na⁺ cells in Na⁺ medium containing 1 mM K_o⁺. To maintain a similar ratio of ouabain molecules/ouabain binding sites, the experiments in MAJ and control cells were carried out at 1 and 10% Hct, respectively.

Measurement of lactate production and cell ATP, ADP and Pi

Cells were incubated at Hct ~ 25% in a medium containing 135 mM NaCl, 10 mM KCl, 1 mM MgCl₂, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4 at 37°C. At different time intervals, a 0.2-ml sample of cell suspension was removed and added to a prechilled centrifuge tube containing 0.4 ml of 8% perchloric acid, and then centrifuged at 1,500 g for 10 min at 4°C. Lactate was determined in a 0.2-ml aliquot of the clear supernatant using the L[+] lactic acid diagnostic kit purchased from Sigma Chemical Co., St. Louis, MO. Lactate production was calculated in millimoles per 1 cell per hour using the Hct of the cell suspension.

Cell ATP and ADP were measured in samples obtained from the same cell suspension where lactate was determined. At each time interval a 0.5-ml aliquot of cell suspension was frozen with acetone-dry ice and then used to measure the ATP-ADP content. 250 μl of the cell suspension were extracted with 360 μl of 6% perchloric acid, neutralized with 125 μl of 0.1 M PO₄K₂ and spun down at 0°C. Aliquots of the supernatant were filtered and applied to an ion-exchange high-performance liquid chromatography (HPLC) column (Partisil SAX) and eluted isocratically using 0.16 M KH₂PO₄ plus 0.1 M KCl, pH 6.5 at room temperature at a flow rate of 1.4 ml/min (=254). Quantitation of ADP and ATP was accomplished using external standards. Cell inorganic phosphate was measured by the method of Baginski (7).

Results

Kinetic properties of the different modes of operation of the Na⁺/K⁺ pump in MAJ cells. We performed several experiments designed to characterize the kinetic properties of the Na⁺/K⁺ pump in MAJ cells.

Na_i⁺-K_o⁺ exchange (normal mode)

Fig. 1 shows the effect of varying Na_i⁺ (salicylate loading) on the net ouabain-sensitive Na⁺ efflux into Na⁺-free medium (choline substitution) when K_o⁺ was 0 or 10 mM. In the presence of 10 mM K_o⁺ and adequate metabolic substrate (inosine, adenine, and P_i), ouabain-sensitive Na⁺ efflux occurred by Na_i⁺-K_o⁺ exchange, the normal mode of operation of the Na⁺/K⁺ pump. The kinetic plot (Hanes plot) of the data, shown in Fig. 1 *b*, yields a straight line when *n* = 3, indicating a stoichiometry of three Na⁺ ions transported per cycle of the pump. The maximum flux in this mode (*J*^{max}), estimated from the slope of the straight line in Fig. 1 *b*, was 60 mmol/1 cell per h, ~ 10 times greater than the maximum pump flux in normal human red cells (8). The apparent dissociation constant for Na_i⁺ (*K*_n⁺) calculated from the intersection of the straight line with the abscissa, was 3.5 mmol/1 cell, similar to the value obtained for normal cells and to that reported by Garay and Garrahan (8).

In experiments in fresh MAJ cells incubated in TEA medium, we determined that the concentration of K_o⁺ required to activate half-maximally the Na⁺ efflux through Na_i⁺-K_o⁺ exchange (*K*_{0.5}^a) was ~ 0.8 mM, a value also similar to that of normal cells (9).

Uncoupled Na⁺ efflux

The ouabain-sensitive Na⁺ efflux into Na⁺- and K⁺-free solutions shown in Fig. 1 *a* is a measure of the Na⁺ efflux mediated through the Na⁺/K⁺ pump that is not coupled to the influx of K⁺ or Na⁺ (10). The kinetic plot of the data at 0 mM external K⁺ produced a straight line when *n* was assumed to be 3 (Fig. 1 *b*), indicating that the uncoupled Na⁺ efflux through the pump, like Na_i⁺-K_o⁺ exchange, has a stoichiometry of three Na⁺ ions transported per cycle of the pump. The maximum rate of the net uncoupled Na⁺ efflux from salicylate loaded MAJ cells was 18

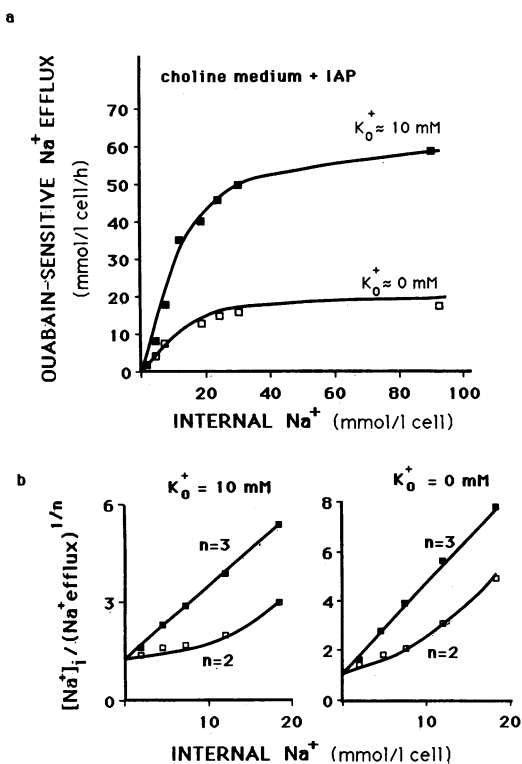


Figure 1. (a) Effect of internal Na^+ on the ouabain-sensitive Na^+ efflux from MAJ cells. Ouabain-sensitive Na^+ efflux from MAJ cells was measured in choline medium at 0 (\square) and 10 (\blacksquare) mM external K^+ . Cell Na^+ was varied with the salicylate loading procedure at 0°C . The external medium contained (mM): 140 or 130 choline chloride; 0 KCl or 10 KCl; 1 MgCl_2 ; 10 glucose; 10 Tris-MOPS, pH 7.4 at 37°C with and without 0.1 ouabain; 1.7 inosine; 3.3 adenine, and 2.4 K-phosphate buffer, pH 7.4. Na^+ efflux was measured by atomic absorption spectrometry. The Hct of the cell suspension was 1–2%. The incubation times were 1, 2, 3, and 6 min in the absence of ouabain and 5 and 25 min in the presence of ouabain. (b) Hanes plots of the ouabain-sensitive Na^+ efflux at 10 and 0 mM K_0^+ . The Eq. 1 was rearranged as follows:

$$\text{Na}_i^+ / ({}^0J_{\text{Na}^+})^{1/3} = [K_n' / ({}^0J_{\text{Na}^+}^{\text{max}})^{1/3}] + [\text{Na}_i^+ / ({}^0J_{\text{Na}^+}^{\text{max}})^{1/3}]$$

The left term of this equation was plotted against Na_i^+ assuming $n = 2$ (\square) and $n = 3$ (\blacksquare). K_n' for internal Na^+ to stimulate Na^+ efflux, obtained from the intercept with the abscissa, was 3.5 mmol/l cell at 10 mM K_0^+ ($\text{Na}_i^+ - \text{K}_0^+$ exchange) and 3 mmol/l cell at 0 mM K_0^+ (uncoupled Na^+ efflux).

mmol/l cell per h, and the K_n' was 3 mmol/l cell, a value that is also in agreement with that reported by Garay and Garrahan (8). We also observed that the uncoupled Na^+ efflux was inhibited by 5 mM external Na^+ (Fig. 2), as previously reported by Garrahan and Glynn (10) and by Sachs (11).

To determine the kinetics of the other operational modes of the pump, MAJ cells were prepared with nystatin to contain either Na^+ or K^+ as the exclusive intracellular cation.

$\text{Na}_o^+ - \text{Na}_i^+$ exchange

In the presence of external Na^+ and absence of external K^+ , the Na^+/K^+ pump exchanges external for internal Na^+ ($\text{Na}_o^+ - \text{Na}_i^+$ exchange) (10). Fig. 2 shows the effect of varying the external Na^+ concentration (choline substitution) on the ouabain-sen-

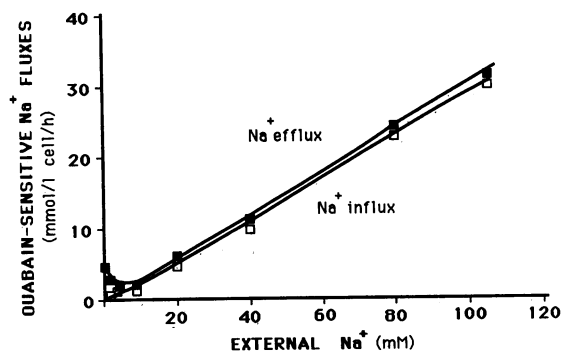


Figure 2. Effect of external Na^+ on the ouabain-sensitive Na^+ influx (\square) and efflux (\blacksquare) from MAJ cells containing only Na^+ into K^+ -free media. Cells were treated with nystatin to contain Na^+ as the only intracellular cation ($\text{K}_i^+ < 0.5$ mmol/l cell). Cell ATP and ADP before the incubation were 0.300 and 0.660 mmol/l cell, respectively. The flux media contained (mM): 0–130 NaCl, 130–0 choline chloride; 1 MgCl_2 ; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C with and without 0.1 ouabain. The Hct of the cell suspension was 1%. The incubation times were 5 and 25 min. $^{22}\text{Na}^+$ was used as a tracer.

sitive Na^+ efflux and influx in MAJ cells containing only Na^+ . Both ouabain-sensitive Na^+ efflux and influx increased linearly with external Na^+ concentrations up to 110 mM and the stoichiometric ratio of the exchange was close to 1. The rate of the exchange when Na_o^+ equaled 140 mM was ~ 30 mmol/l cell per h, ~ 20 times higher than in normal cells (rate of $\text{Na}_o^+ - \text{Na}_i^+$ exchange in high- Na^+ control cells at 140 mM $\text{Na}_o^+ = 1.4 \pm 0.1$ mmol/l cell per h). The high value of the maximum rate of the exchange in MAJ cells loaded with nystatin is probably due to the higher cellular ADP/ATP ratio observed in these cells (ADP and ATP values in MAJ and control cells loaded with nystatin to contain 100 mmol/l cell Na^+ : [mmol/l cell]: MAJ cells: ADP 0.650 ± 0.1 , ATP 0.300 ± 0.1 ; control cells: ADP 0.380 ± 0.08 , ATP 0.860 ± 0.15) (12–14). Measuring the unidirectional $^{22}\text{Na}^+$ influx in fresh MAJ cells we showed that the $\text{Na}_o^+ - \text{Na}_i^+$ exchange was inhibited by K_0^+ with a $K_{0.5}^i$ of ~ 1 mM, similar to the value reported for normal cells by Sachs (11). Fig. 3 shows the effect of external K^+ on the ouabain-sensitive Na^+ efflux and influx in MAJ cells loaded with Na^+ by the nystatin procedure. Under these conditions, with glucose as the only metabolic substrate, MAJ cells rapidly become depleted of ATP. The concentration of external K^+ required to inhibit both Na^+ influx and Na^+ efflux half maximally ($K_{0.5}^i$) was ~ 1 mM. This indicates that cells partially depleted of ATP incubated in the absence of adequate metabolic substrate, were able to transport Na^+ through $\text{Na}_i^+ - \text{Na}_o^+$ exchange but not to shift to $\text{Na}_i^+ - \text{K}_0^+$ exchange, which requires the hydrolysis of ATP. The ouabain-sensitive Na^+ efflux at 10 mM K_0^+ (~ 5 mmol/l cell per h, Fig. 3) might represent $\text{Na}_i^+ - \text{K}_0^+$ exchange at $< 10\%$ of the maximal rate due to ATP depletion.

$\text{Na}_o^+ - \text{K}_i^+$ exchange (reversal mode)

Garrahan and Glynn (15) showed that an ouabain-sensitive incorporation of ^{32}P i into ATP occurred when human red cell ghosts containing mainly K^+ were incubated in a medium containing only Na^+ . Glynn and Lew (16) and Glynn et al. (17) observed that ouabain-sensitive synthesis of ATP associated with both ouabain-sensitive K^+ efflux and Na^+ influx also occurred

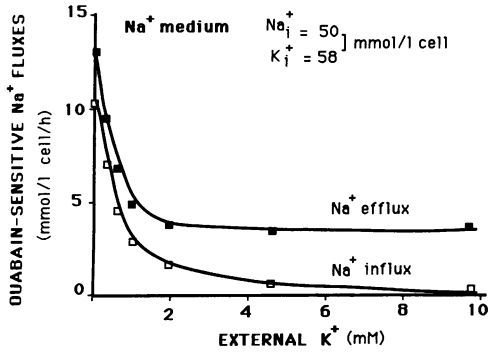


Figure 3. Effect of external K^+ on the ouabain-sensitive Na^+ influx (\square) and efflux (\blacksquare) from metabolically depleted MAJ cells containing high Na^+ . Cells were treated with nystatin to contain 50 and 58 mmol/l cell of Na^+ and K^+ , respectively. ATP before the incubation was < 0.05 mmol/l cell. The flux media contained (mM): 140–130 NaCl, 0–10 KCl, 1 $MgCl_2$; 10 glucose; 10 Tris-MOPS pH 7.4 at $37^\circ C$ with and without 0.1 ouabain. The Hct of the cell suspension was 1%. The incubation times were 5 and 25 min. $^{22}Na^+$ was used as a tracer. The $K_{0.5}$ for external K^+ to inhibit both fluxes was ~ 1 mM.

when starved human red cells with high K^+ content were incubated in a medium containing only Na^+ . Moreover, both ouabain-sensitive ATP synthesis and K^+ efflux increased linearly with the extracellular Na^+ concentration and were inhibited by small concentrations of K^+ in the media (16, 17). These experiments indicate that the Na^+/K^+ pump may run backwards with reversal of the pump cycle (reversal mode). Fig. 4 shows the ouabain-sensitive K^+ ($^{86}Rb^+$) efflux and Na^+ influx measured in cells containing only K^+ as a function of the external Na^+ concentration in K^+ -free medium. Both Na^+ influx and K^+ efflux saturated at about 3.2 mmol/l cell per h, were stimulated by external Na^+ and were half maximal when Na^+_o was ~ 30 mM. The stoichiometric ratio of the exchange was 1 except at low Na^+_o when K^+ efflux probably occurred by “uncoupled” K^+ efflux or by another mechanism. When influx and efflux of Na^+ were measured in fresh MAJ cells incubated in a medium containing 140 mM Na^+ but no K^+ , we observed that there was an excess of ouabain-sensitive influx over efflux and that the difference balanced the ouabain-sensitive K^+ efflux (Fig. 7). This ouabain-

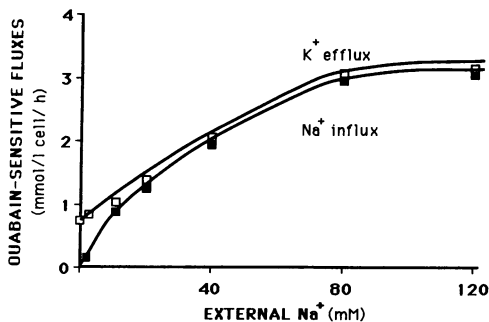


Figure 4. Effect of external Na^+ on the ouabain-sensitive Na^+ influx (\blacksquare) and K^+ efflux (\square) from MAJ cells containing only K^+ into K^+ -free media. Cells were treated with nystatin to contain K^+ as the exclusive intracellular cation. Intracellular Na^+ at the end of the loading was ~ 0.5 mmol/l cell. Experimental conditions were identical to those in Fig. 2. $^{22}Na^+$ and $^{86}Rb^+$ were used as tracers.

sensitive exchange of external Na^+ for internal K^+ was inhibited by external K^+ with a $K_{0.5}^i \sim 1$ mM, a value similar to the concentration of extracellular K^+ at which, under similar conditions, ouabain-sensitive Na^+ efflux, K^+ influx, and Na^+ influx are all half maximal. This value is also in agreement with the concentration of extracellular K^+ reported by Glynn and Lew to inhibit half-maximally the ouabain-sensitive synthesis of ATP in red cell ghosts incubated in media containing only Na^+ (16). Thus, we conclude that cation fluxes mediated by the pump operating backwards (reversal mode) were measurable in MAJ cells, even though the cells were not depleted of ATP. We plan to measure in future experiments the qualitative relationship between the rates of K^+ and Na^+ movement through the pump in the reversal mode and the rate of ATP synthesis.

$K^+-K^+_o$ exchange and “uncoupled” K^+ efflux

The ouabain-sensitive K^+ (^{86}Rb) influx and efflux were measured in cells containing only K^+ as a function of the external K^+ concentration in Na^+ -free medium (choline substitution). Fig. 5 shows that a sizable ouabain-sensitive K^+ efflux (≈ 1.4 mmol/l cell per h) was present into K^+ -free solutions (uncoupled K^+ efflux) and that both K^+ efflux and influx were stimulated by external K^+ . These fluxes represent the $K^+_i-K^+_o$ exchange mediated by the Na^+/K^+ pump. The maximal rate of the ouabain-sensitive K^+ efflux was 6.6 mmol/l cell per h, a value 10 times higher than that reported by Simons (18). The $K_{0.5}^a$ for external K^+ to stimulate the exchange was 0.7 mM. An interesting observation in MAJ cells was that at saturating K^+_o the ratio of the K^+ efflux and influx by the $K^+_i-K^+_o$ exchange was not 1, as reported by Simons (18), but between 1.5 and 2. This discrepancy might indicate that under these conditions the Na^+/K^+ pump mediates an uncoupled K^+ efflux and a 1:1 $K^+_i-K^+_o$ exchange or, alternatively, that the stoichiometry of the $K^+_i-K^+_o$ exchange is 3:2. Ouabain-sensitive $K^+_i-K^+_o$ exchange depends on the inorganic phosphate content of the cell (17, 18). Since the dependence of ouabain-sensitive $K^+_i-K^+_o$ exchange on inorganic phosphate was not studied in our experiments, it is possible that maximal rate and stoichiometry of this mode of the pump could be affected by internal inorganic phosphate.

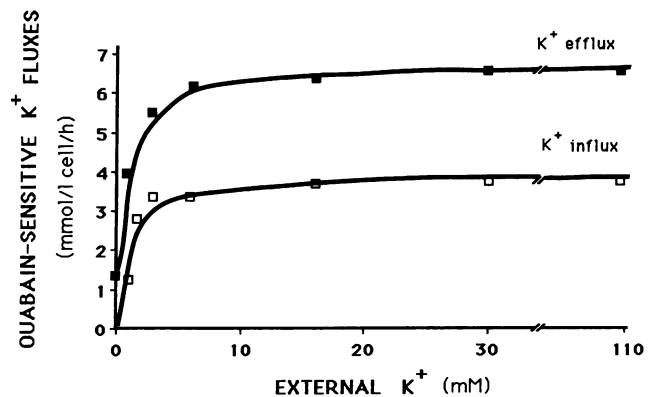


Figure 5. Effect of external K^+ on the ouabain-sensitive K^+ influx (\square) and efflux (\blacksquare) from MAJ cells containing only K^+ into Na^+ -free media. The cells were the same as in Fig. 4. The flux media contained (mM): 0–130 KCl, 130–0 choline chloride; 1 $MgCl_2$; 10 glucose; 10 Tris-MOPS pH 7.4 at $37^\circ C$ with and without 0.1 ouabain. Other experimental conditions were similar to Figs. 2 and 4. $^{86}Rb^+$ was used as a tracer.

The results of our kinetic experiments confirm that MAJ red cells have about 10 times more Na^+/K^+ pumps than normal cells and indicate that these pumps are, in most ways, similar to the pumps in normal cells.

Physiological effects of the high density of Na^+/K^+ pumps in MAJ cells. In order to understand the physiological consequences of the increased density of Na^+/K^+ pumps in MAJ cells we studied their cation composition, the unidirectional Na^+ and K^+ fluxes in fresh cells and the relationship between the Na^+/K^+ pump and the cell metabolism.

Cation composition of MAJ cells

Table I shows the internal content of cations, ATP, ADP, and Pi of MAJ and normal cells. The most striking abnormalities of MAJ cells are their very low intracellular Na^+ (2.2 mmol/l cell) and increased intracellular K^+ (113 mmol/l cell). The total content of cations, ATP, ADP, and Pi do not differ significantly from the values observed in normal control cells.

Na^+ and K^+ transport in MAJ and control cells incubated in vitro in a plasma-like medium

To investigate how the increased density of pumps influences the intracellular cation composition, we measured the unidirectional cation fluxes in fresh MAJ and control cells in a medium with Na^+ and K^+ concentration similar to human plasma, i.e., 140 mM Na_o^+ and 4 mM K_o^+ (the cation composition of MAJ plasma was similar to that of control subjects). Under these physiological conditions the net ouabain-sensitive and the net ouabain-insensitive Na^+ fluxes were similar in MAJ ($\text{Na}_i^+ = 2.2$ mmol/l cell) and control fresh cells ($\text{Na}_i^+ = 8.5$ mmol/l cell): (mmol/l cell per h): net ouabain-sensitive Na^+ flux (outwardly directed): MAJ = 1.3 ± 0.02 , control = 1.4 ± 0.15 ; net ouabain-insensitive Na^+ flux (inwardly directed): MAJ = 0.8 ± 0.01 , control = 0.8 ± 0.01 . The difference between the outwardly and inwardly directed net Na^+ fluxes can probably be accounted for by the lack in the incubation medium of bicarbonate, which mediates a net inwardly directed, ouabain-insensitive Na-carbonate flux mediated by capnophorin, the anion exchange protein (19). At physiological concentration of bicarbonate the net inwardly directed movement of Na through the Na-carbonate pathway would be in the order of 1 mmol/l cell per h, as estimated from the data reported by Funder (19). Since the active (ouabain sensitive) Na^+ efflux depends both on the number of transport sites and the turnover rate of the pump, which is controlled, in part, by the intracellular Na^+ , our data indicate that the low intra-

cellular Na^+ in MAJ cells compensates for the increased density of pumps so that the uphill Na^+ efflux equals the downhill Na^+ influx.

The effect of external K^+ on the ouabain-sensitive Na^+ efflux from fresh MAJ cells

In fresh human red cells (intracellular $\text{Na}^+ \sim 8\text{--}10$ mmol/l cell), external K^+ stimulates both ouabain-sensitive Na^+ efflux and K^+ influx (20, 21). We found that the ouabain-sensitive Na^+ efflux from fresh MAJ cells ($\text{Na}_i^+ = 2.2$ mmol/l cell) into 140 mM Na_o^+ was not stimulated by external K^+ , although external K^+ did stimulate the ouabain-sensitive K^+ influx (Fig. 6 a) with a $K_{0.5}$ similar to that of control cells at the same intracellular Na^+ (insets Fig. 6 a and b). To investigate whether this apparently abnormal behavior of the ouabain-sensitive Na^+ efflux could be due to the low intracellular Na^+ of MAJ cells, we measured the ouabain-sensitive Na^+ efflux and K^+ influx in control cells treated with nystatin to contain low intracellular Na^+ (~ 2 mmol/l cell). Similarly to MAJ cells, the ouabain-sensitive Na^+ efflux from low- Na^+ control cells into 140 mM Na_o^+ was not stimulated by external K^+ (Fig. 6 b). However, the substitution of the ouabain-sensitive Na^+ efflux by external K_o^+ in both MAJ and low- Na^+ control cells (Table II, see also Fig. 8). Table II shows that the ouabain-sensitive Na^+ efflux observed in Na^+ -containing me-

Table I. Composition of MAJ Cells

	MAJ	Control
	mmol/l cell	mmol/l cell
Na^+	2.17 ± 0.1	8.3 ± 0.2
K^+	113 ± 2	98 ± 3
ATP	0.97 ± 0.15	1.2 ± 0.2
ADP	0.224 ± 0.02	0.170 ± 0.02
Pi	0.58 ± 0.1	0.48 ± 0.2

The values were obtained from MAJ and control fresh cells. Intracellular Na and K were measured by atomic absorption spectrometry and ATP, ADP, and Pi as described in Methods. Values are mean \pm SEM of three determinations.

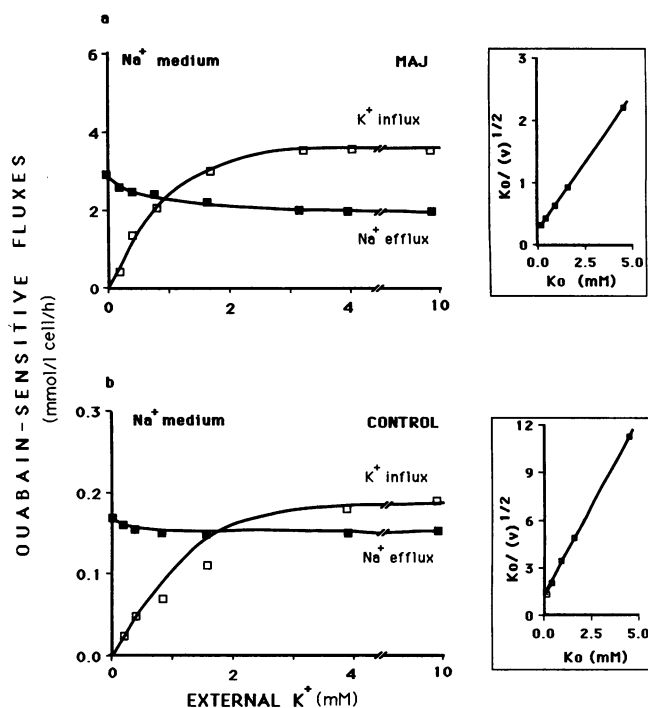


Figure 6. Effect of external K^+ on the ouabain-sensitive Na^+ efflux (■) and K^+ influx (□) in MAJ (a) and control (b) cells in Na^+ medium. Cell Na^+ was reduced to ~ 2 mmol/l cell in control cells with the nystatin method. The media contained (mM): 140–130 NaCl; 0–10 KCl; 1 MgCl_2 ; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C with and without 0.1 ouabain. $^{22}\text{Na}^+$ and $^{86}\text{Rb}^+$ were used as tracer. The Hct of the flux suspensions were 1% for the efflux and 5% for the influx. The incubation times were 2 and 7 min in MAJ cells and 5 and 25 min in control cells. (Insets) The Hanes plots of the ouabain-sensitive K^+ influx vs. K_o^+ . The $K_{0.5}$ for K_o^+ to stimulate the ouabain-sensitive K^+ influx (intercept with the abscissa) was ~ 0.6 mM in both MAJ and control cells.

Table II. The Effect of External K^+ on the Ouabain-sensitive Na^+ Efflux from Cells with Low Na^+ Content

Medium	Ouabain-sensitive Na^+ efflux			
	MAJ		Control	
	0 mM K_0^+	10 mM K_0^+	0 mM K_0^+	10 mM K_0^+
	mmol/l cell/h		mmol/l cell/h	
140 mM Na^+	2.8±0.2	2±0.1	0.198±0.01	0.186±0.01
0 mM Na^+ (140 mM TEA)	0.250±0.02	1.97±0.1	0.004±0.001	0.100±0.01

Cell Na^+ was ~ 2 mmol/l cell in both MAJ and control cells. The Na^+ content of control cells was reduced with nystatin. The incubation media contained (mM): 140–130 NaCl or 140–130 TEA; 0 or 10 KCl; 1 MgCl₂; 10 glucose; 10 Tris-MOPS, pH 7.4 at 37°C with and without 0.1 ouabain. Incubation times were 2 and 7 min for MAJ cells in the absence of ouabain and 2 and 25 for all the other fluxes.

dium at 0 mM K_0^+ was absent in Na^+ - (and K^+) free medium (TEA substitution) indicating that in the presence of Na_0^+ and absence of K_0^+ the ouabain-sensitive Na^+ efflux was driven by Na_i^+ - Na_0^+ exchange. The data shown in Table II suggest that when either MAJ cells or normal cells containing ~ 2 mmol/l cell Na^+ are incubated in Na^+ medium, the almost similar values of the Na^+ efflux through the Na_i^+ - Na_0^+ (0 mM K_0^+) and Na_i^+ - K_0^+ exchanges (10 mM K_0^+) result in the observed lack of stimulation of the ouabain-sensitive Na^+ efflux by external K^+ , as we will discuss below.

The unidirectional Na^+ and K^+ fluxes in fresh MAJ cells

Fig. 7 shows the four unidirectional ouabain-sensitive Na^+ and K^+ fluxes in MAJ cells, measured in 140 mM Na_0^+ as a function of the external K^+ concentration. The ouabain-sensitive Na^+ influx was maximal in the absence of external K^+ and was inhibited as K^+ was increased in the flux medium ($K_{0.5}^i \approx 1$ mM). Furthermore, in the absence of external K^+ the ratio of the ouabain-sensitive Na^+ influx and efflux was ~ 2, indicating that a portion of the Na^+ influx was driven by the exchange for internal K^+ (reversal, Na_0^+ - K_i^+ exchange). Fig. 7 also shows that a large K^+ efflux was present through the whole range of external K^+ concentrations. The reversal mode accounts for the K^+ efflux observed at low but not at high K_0^+ , since this mode is inhibited by external K^+ (Fig. 9). The K^+ efflux observed as K_0^+ increases could be accounted for by a 1:1 K_i^+ - K_0^+ exchange and an uncoupled K^+ efflux that is stimulated by K_0^+ or, alternatively, by a 3:2 K_i^+ - K_0^+ exchange.

We investigated the ability of fresh MAJ cells to carry out Na^+ and K^+ fluxes through the different operational modes of the Na^+ / K^+ pump. To estimate the fluxes through the normal mode (Na_i^+ - K_0^+ exchange) we measured the ouabain-sensitive unidirectional Na^+ and K^+ fluxes in Na^+ -free solutions in which neither Na_i^+ - Na_0^+ exchange nor reversal mode can take place. However, when Na_0^+ was replaced by choline, we observed that the values of the ouabain-sensitive Na^+ efflux from MAJ cells were similar at low and high K_0^+ . This observation might be accounted for by uncoupled Na^+ efflux present at low K_0^+ and by a greater apparent affinity for K_0^+ exhibited by the Na^+ / K^+ pump when choline replaces Na^+ in the flux media (11). From the values of $K_{0.5}^a$ and $^1J_{K^+}^{max}$ derived from the kinetic plot

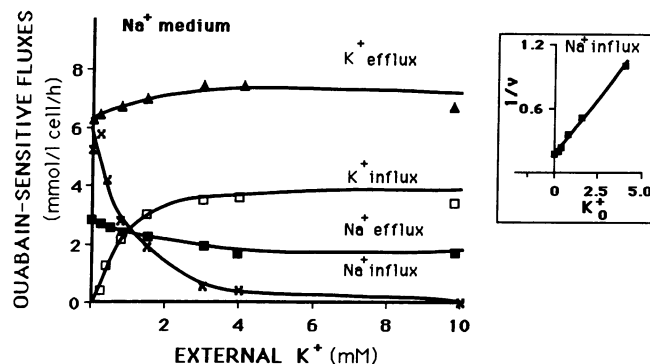


Figure 7. Effect of external K^+ on the ouabain-sensitive unidirectional Na^+ and K^+ fluxes from MAJ fresh cells into Na^+ medium. Cell Na^+ was 2.3 mmol/l cell. The flux media contained (mM): 140–130 NaCl; 0–10 KCl; 1 MgCl₂; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C with and without 0.1 ouabain. The Hct of the cell suspension was 1% for the efflux and 5% for the influx. The incubation times for the fluxes without and with ouabain were 2 and 7 min and 5 and 25 min, respectively. $^{22}Na^+$ and $^{86}Rb^+$ were used as tracers. (■) ouabain-sensitive Na^+ efflux; (×) ouabain-sensitive Na^+ influx; (▲) ouabain-sensitive K^+ efflux; (□) ouabain-sensitive K^+ influx. (Inset) Dixon plot of the ouabain-sensitive Na^+ influx vs. K_0^+ . The $K_{0.5}^i$ for K_0^+ to inhibit the ouabain-sensitive Na^+ influx (interception with the abscissa) was ~ 0.8 mM.

shown in the inset to Fig. 8 b and from the value of K_0^+ measured at the end of the flux period in the nominally 0 mM K_0^+ media (20 μ M), we estimated a Na^+ efflux through Na_i^+ - K_0^+ exchange of ~ 0.8 mmol/l cell per h, which accounts for ~ 50% of the ouabain-sensitive Na^+ efflux at nominally 0 mM K_0^+ in choline medium. When Na_0^+ was substituted by TEA, the ouabain-sensitive Na^+ efflux from MAJ cells at nominally 0 mM K_0^+ was less than one-tenth of the value at 10 mM K_0^+ (Fig. 8 a). Similar results were obtained in normal red cells containing low internal Na^+ . Thus, when low- Na^+ cells are incubated in TEA medium external K^+ stimulates the ouabain-sensitive Na^+ efflux (Na_i^+ - K_0^+ exchange) in both MAJ and normal cells. This is so because TEA, a competitive inhibitor of external K_0^+ on the Na^+ / K^+ pump (9), behaves like extracellular Na^+ decreasing the apparent affinity for K_0^+ and, perhaps, reducing the uncoupled Na^+ efflux. We thus assumed that the K_0^+ -stimulated, ouabain-sensitive Na^+ efflux into TEA medium represented the pump mediated Na_i^+ - K_0^+ exchange. Then, with the values of the unidirectional Na^+ and K^+ fluxes measured in Na^+ medium as a function of external K^+ , we calculated the fluxes through the different operational modes at each external K^+ concentration as follows. (a) The ouabain-sensitive Na^+ efflux in Na^+ medium minus the ouabain-sensitive Na^+ efflux in TEA medium (taken as the component of the efflux carried out by Na_i^+ - K_0^+ exchange), was assumed to represent the Na^+ efflux by Na_i^+ - Na_0^+ exchange; since the stoichiometric ratio of this mode is 1, it also represented the Na^+ influx through the exchange pathway. (b) The total ouabain-sensitive Na^+ influx minus the ouabain-sensitive Na^+ influx mediated by Na_i^+ - Na_0^+ exchange was taken to equal the Na^+ influx (carried out by the reversal Na_0^+ - K_i^+ exchange). Fig. 9 shows that, in 140 mM Na^+ medium, external K^+ stimulated the Na_i^+ - K_0^+ exchange and inhibited both the Na_0^+ - Na_i^+ exchange and the reversal mode. Similar calculations were made for the K^+ fluxes in Na^+ medium and for the different modes in choline and in TEA media (not shown).

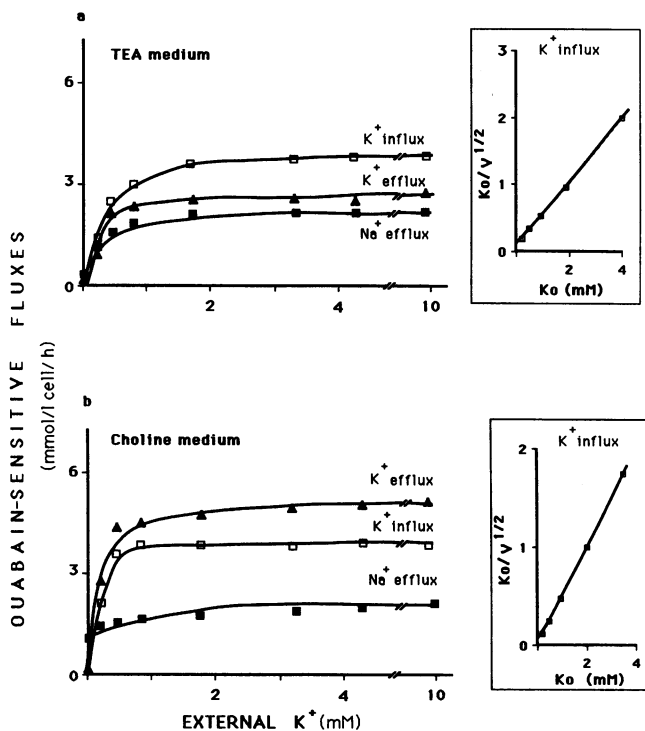


Figure 8. Effect of external K^+ on the ouabain-sensitive unidirectional Na^+ and K^+ fluxes in MAJ fresh cells into TEA medium (a) and choline medium (b). Cell Na^+ was 2.3 mmol/l cell. The flux media contained (mM): 140–130 TEA (a) or choline chloride (b); 0–10 KCl; 1 $MgCl_2$; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C with and without 0.1 ouabain. The hematocrit of the cell suspension was 1% for the efflux and 5% for the influx. All other conditions were identical to those in Fig. 7. (■) ouabain-sensitive Na^+ efflux; (▲) ouabain-sensitive K^+ efflux; (□) ouabain-sensitive K^+ influx. (Insets) The Hanes plots of the ouabain-sensitive K^+ influx vs. K_o^+ in TEA (a) and choline (b) media. The $K_{0.5}$ for K_o^+ to stimulate the ouabain-sensitive K^+ influx was ~ 0.5 mM in TEA and 0.03 mM in choline medium.

$K_{0.5}^i$ for ouabain of MAJ cells

DeLuise and Flier (1) found that MAJ cells have a reduced $K_{0.5}^i$ for ouabain as compared with normal cells. Since it is known

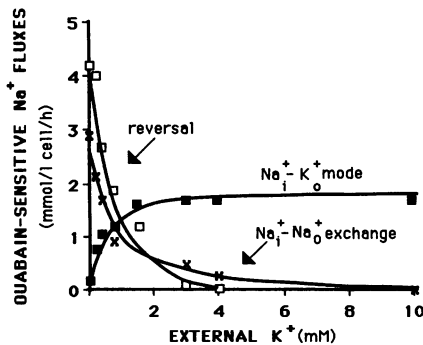


Figure 9. Effect of external K^+ on the different modes of operation of the Na^+/K^+ pump in Na^+ medium. From the ouabain-sensitive unidirectional Na^+ and K^+ fluxes into Na^+ medium shown in Fig. 7 and from the ouabain-sensitive unidirectional Na^+ efflux into TEA medium shown in Fig. 8, the Na^+ fluxes mediated through the Na^+-K^+ mode (■), the Na^+-Na^+ exchange (x) and the K^+-Na^+ exchanges were calculated as described in the text.

that the rate of glycoside binding to red cells depends on the turnover rate of the Na^+/K^+ pump and is therefore modulated by internal Na^+ (22), we investigated whether the decreased $K_{0.5}^i$ for ouabain in MAJ cells could be accounted for by their low intracellular Na^+ . We measured the $K_{0.5}^i$ for ouabain to inhibit the ^{86}Rb influx in MAJ and control cells containing either normal or low Na^+ . We used a 10-fold higher hematocrit for control cells to have a similar ratio of ouabain molecules in the medium to ouabain-binding sites. Fig. 10 shows that the $K_{0.5}^i$ for ouabain was reduced in MAJ cells only if compared with fresh control cells that contained a threefold higher intracellular Na^+ (2.3 vs. 8.5 mmol/l cell). When the Na^+ content of the control cells was reduced to 2 mmol/l cell by the nystatin procedure, no difference was found in the kinetics of Rb^+ influx inhibition by ouabain (Fig. 10).

Effect of activation of the Na^+/K^+ pump on lactate production and ATP levels in MAJ cells

The metabolism of glucose through the Embden-Meyerhof glycolytic pathway is the exclusive source of ATP in mature red cells. Since the glycolytic rate cannot be increased more than threefold (23), it is reasonable to suspect that the availability of ATP in MAJ cells would be rate limiting for the operation of the Na^+/K^+ pump. Fig. 11 shows that when glucose was the only metabolic substrate present in the flux medium, Na^+ -loaded MAJ cells could not maintain their ouabain-sensitive lactic acid production and ATP level. In contrast, addition to the incubation medium of the metabolic substrates inosine and adenine as well as inorganic phosphate (IAP) (which provides phosphorylated substrate to the lower part of the glycolytic reaction sequence), not only resulted in a 12-fold increase in the lactic acid production but also avoided ATP depletion (Fig. 11). The inset in Fig. 11 shows the effect of IAP medium on the ouabain-sensitive Na^+ efflux from cells loaded to contain 30 mmol/l cell intracellular Na^+ . These results indicate that the energy required for transport work can be provided by glucose metabolism only within a limited range of Na^+/K^+ ATPase activity.

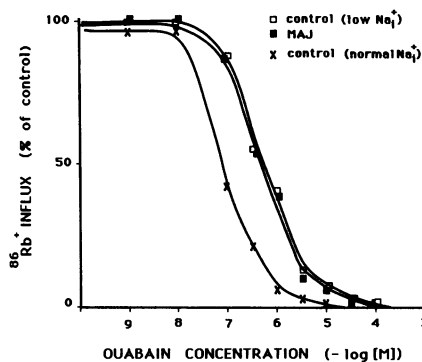


Figure 10. Effect of increasing concentrations of ouabain on the K^+ influx into MAJ fresh cells (■) and into control fresh (x) and low- Na^+ cells (□). Cell Na^+ (mmol/l cell) was 2.3 in MAJ and control low- Na^+ cells and 8.3 in control fresh cells. Control low- Na^+ cells were prepared with nystatin. The flux medium contained (mM): 135 NaCl; 1 mM KCl; 1 $MgCl_2$; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C and $0-10^{-3}$ ouabain. The Hct of the cell suspension was 1% in MAJ cells and 10% in control cells. The incubation time was 10 min. $^{86}Rb^+$ was used as a tracer. Fluxes are expressed as % of the value observed in the absence of ouabain.

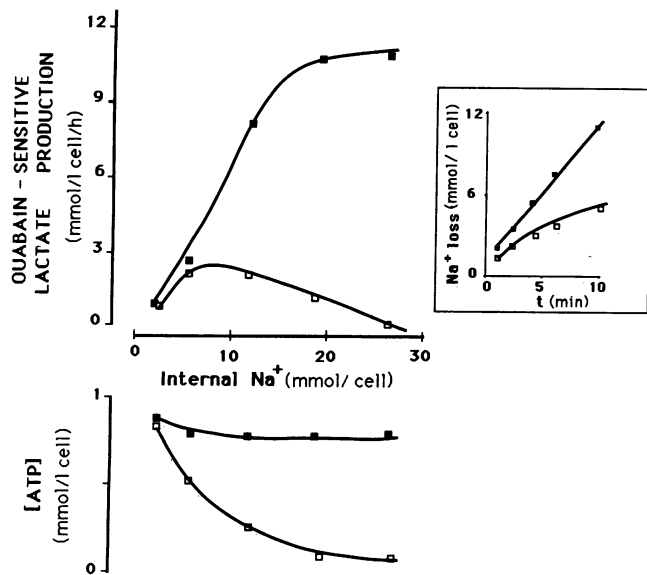


Figure 11. Effect of inosine, adenine, and inorganic phosphate (IAP) on the cell ATP levels, ouabain-sensitive lactate production and Na⁺ efflux in MAJ Na⁺-loaded cells. Cell Na⁺ was varied with the salicylate loading procedure. Lactate and ATP were measured in media containing (mM): 135 NaCl; 10 mM KCl; 1 MgCl₂; 10 glucose; 10 Tris-MOPS pH 7.4 at 37°C with and without 0.1 ouabain in the presence (■) and absence (□) of 1.7 inosine; 3.3 adenine; 2.4 K-phosphate buffer (IAP). The Hct of the cell suspension was 25%. The incubation times were 1, 2, 4, 6, and 12 min. ATP levels were measured after 5 min. (Inset) The effect of IAP medium on the ouabain-sensitive Na⁺ loss, measured as accumulation of Na⁺ into Na⁺-free medium, from MAJ cells loaded to contain 30 mmol/l cell Na⁺. The external medium contained (mM): 130 choline chloride; 10 KCl; 1 MgCl₂; 10 glucose; 10 Tris-MOPS, pH 7.4 at 37°C with and without 0.1 ouabain; with (■) and without (□) IAP.

Discussion

Kinetic properties of the Na⁺/K⁺ pumps in MAJ cells. The data presented in this paper are consistent with the conclusion that the Na⁺/K⁺ pumps in MAJ red cells are similar to the pumps in the red cell membranes of normal human subjects. The maximum turnover numbers and the concentrations of internal and external K⁺ and Na⁺ required to activate the several modes of operation of the pump are indistinguishable in MAJ and normal cells (Table III). The results do reveal certain kinetic characteristics of Na⁺ and K⁺ transport in both MAJ and normal cells that deserve further investigation.

Activation of Na⁺ efflux by Na_i⁺

Ouabain-sensitive Na⁺ efflux from human red cells moves through three modes of the Na⁺-K⁺ pump; Na_i⁺-K_o⁺ exchange (normal mode), Na_i⁺-Na_o⁺ exchange and an uncoupled efflux of Na⁺ that occurs in the absence of both external Na⁺ and K⁺. The relatively large magnitude of these fluxes in MAJ cells allows for quantitative assessment of the maximum transport rates of these three modes (see Figs. 1 and 2 and Table III). Garay and Garrahan (8) have shown that the activation of ouabain-sensitive Na⁺ efflux through Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchange modes by Na_i⁺ can be described by the equation:

$${}^0J_{Na^+} = {}^0J_{Na^+}^{max} / (1 + K'_{Na} / Na_i^+)^3 \quad (\text{Eq. 1})$$

at all values of K_o⁺. The value of ⁰J_{Na⁺}^{max} is generally different for

Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchange modes. It is noteworthy that the values for K'_{Na} for Na_i⁺-K_o⁺ exchange and uncoupled Na⁺ efflux in MAJ and normal cells are the same (~ 3.5 mmol/l cell) when calculated from this equation, a value similar to that reported by Garay and Garrahan (8) for the Na_i⁺-Na_o⁺ exchange.

Activation and inhibition of pump Na⁺ efflux by K_o⁺

External K⁺ activates Na⁺ efflux by Na_i⁺-K_o⁺ exchange but inhibits Na⁺ efflux by Na_i⁺-Na_o⁺ exchange. Thus, depending on the relative magnitudes of Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchanges, external K⁺ can stimulate, inhibit, or leave unchanged ouabain-sensitive Na⁺ efflux. All three situations were encountered in the experiments reported in this paper.

When internal Na⁺ is ~ 2 mmol/l cell and Na_o⁺ is 140 mM, addition of K⁺ to the medium does not affect ouabain-sensitive Na⁺ efflux in MAJ or normal cells (see Figs. 6, and 7). Apparently, under these conditions, activation of Na_i⁺-K_o⁺ exchange just balances inhibition of Na_i⁺-Na_o⁺ exchange by K_o⁺. This apparent insensitivity of ouabain-sensitive Na⁺ efflux to external K⁺ is not observed at higher values of Na_i⁺ in normal cells (11). Under these conditions, addition of K_o⁺ to a medium containing 140 mM Na⁺ produces an increase in ouabain-sensitive Na⁺ efflux. The explanation for this observed change in the relative magnitudes of Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchange as a function of Na_i⁺ is not clear. The result apparently conflicts with the view that stoichiometry and affinity for internal Na⁺ are similar for the Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ modes of operation of the pump as suggested above. If stoichiometry and affinity for internal Na⁺ were identical for Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchanges, the relative magnitudes of the two modes should be independent of Na_i⁺ (see Eq. 1). It is possible that the maximum transport rates for Na_i⁺-K_o⁺ and Na_i⁺-Na_o⁺ exchanges are not constant, but rather depend on Na_i⁺ or the ratio of Na_i⁺ to K_o⁺. In any case, further experiments are needed to define the kinetic basis of the apparent insensitivity of ouabain-sensitive Na⁺ efflux to K_o⁺ in MAJ and normal cells containing ~ 2 mmol Na_i⁺/1 cell and suspended in a medium containing Na⁺ (140 mM).

When MAJ cells containing 50–60 mmol/l cell Na⁺ and K⁺ are incubated in a medium with 140 mM Na⁺, addition of K⁺ to the medium inhibits both Na⁺ influx and efflux (see Fig. 3). The explanation for this apparently anomalous result can be found in Fig. 11. When glucose is the only metabolic substrate in the medium, addition of K_o⁺ activates the normal mode of the pump to such an extent that glycolysis cannot keep up and ATP concentration falls, depriving the pump of essential fuel for Na_i⁺-K_o⁺ exchange. Under these conditions, ouabain-sensitive Na⁺ efflux is mediated entirely by Na_i⁺-Na_o⁺ exchange, which is inhibited by K_o⁺.

When MAJ and normal cells containing a low Na_i (~ 2 mmol/l cell) were incubated in media not containing Na⁺, the effect of adding K_o⁺ on ouabain-sensitive Na⁺ efflux depended on the nature of the ion substituting for Na_o⁺ (Fig. 8). When the ion was choline, K_o⁺ had no effect on Na⁺ efflux, but when the ion was TEA, K_o⁺ stimulated Na⁺ efflux. This difference may have been due to the presence of Na_i⁺-K_o⁺ exchange and uncoupled Na⁺ efflux in cells incubated in choline medium in the absence of K_o⁺. Under these conditions, the affinity of the pump for K_o⁺ is extremely high (see Fig. 8 a) (11), and the accumulation of K_o⁺ leaked from the cells into the putatively K⁺-free medium (20 μM at the end of the flux period) may have been sufficient to activate the normal mode of the pump. In the presence of TEA in the medium, the apparent affinity of the pump for K_o⁺

Table III. Kinetic Properties of MAJ Pumps

Mode	Units	Normal	ⁱ J/ ^o J	MAJ	ⁱ J/ ^o J	MAJ/Normal
Na_i⁺/K_o⁺ exchange						
Maximum flux	mmol/1 cell/h	6	2/3	60	2/3	10
K _n ⁺ (Na _i ⁺)	mmol/1 cell	3.5		3.5		
K _{0.5} ^a (K _o) TEA	mM	0.8		0.6		
K _{0.5} ^a (K _o) Choline	mM	0.030		0.034		
Uncoupl. Na⁺ efflux						
Maximum flux	mmol/1 cell/h	1.2		18		15
K _n ⁺ (Na _i ⁺)	mmol/1 cell	3.2		3.0		
Na_i⁺-Na_o⁺ exchange						
Highest flux	mmol/1 cell/h	1.5	1/1	30	1/1	20
K _{0.5} ^a (Na _o ⁺)	mM	>150		>150		
K _{0.5} ⁱ (K _o ⁺)	mM	1		1		
K_i⁺-K_o⁺ exchange						
Maximum flux	mmol/1 cell/h	—	—	6.6	2/3	
K _{0.5} ^a (K _o ⁺)	mM	—		0.7		
K_i⁺-Na_o⁺ exchange						
Maximum flux	mmol/1 cell/h	0.15	1/1	3.2	1/1	20
K _{0.5} ^a (Na _o ⁺)	mM	30		30		

ⁱJ, influx; ^oJ, efflux.

is not as high because of competition between K⁺ and TEA (9). Under these conditions, the concentration of K_o⁺ in the putatively K⁺-free medium is not sufficient to activate appreciably the normal mode of operation of the pump, and addition of K⁺ to the medium increases Na⁺ efflux by Na_i⁺-K_o⁺ exchange (see Fig. 8). We estimated that at 20 μM external K⁺, the Na_i⁺-K_o⁺ exchange would operate at ~ 40% of maximal rate, a value that accounts for one-half of the ouabain-sensitive Na⁺ efflux measured in choline medium at nominally 0 mM K_o⁺. The additional Na⁺ efflux under these conditions could be mediated by uncoupled ouabain-sensitive Na⁺ efflux. TEA but not choline may inhibit the uncoupled ouabain-sensitive Na⁺ efflux observed in the absence of external Na⁺ and K⁺. Since the Albers-Post model of the Na⁺/K⁺ pump reaction mechanism, modified to account for the uncoupled Na⁺ efflux, predicts an uncoupled ouabain-sensitive K⁺ efflux (24), with characteristics similar to those of the uncoupled Na⁺ efflux, this proposal predicts that TEA might also inhibit the uncoupled K⁺ efflux. This could explain the observed reduction of the ouabain-sensitive K⁺ efflux from MAJ cells incubated in TEA medium (see Fig. 8). It is tempting to speculate that TEA would inhibit the pathway of the Albers-Post sequence that is common to both uncoupled fluxes, i.e., the spontaneous dephosphorylation of E₂P that takes place in the absence of external Na⁺ and K⁺. We plan to test this possibility in the future.

Activation of K⁺ efflux by K_o⁺

An impressive feature of the ouabain-sensitive Na⁺ and K⁺ fluxes in fresh MAJ cells incubated in plasmalike medium is the large magnitude of the K⁺ efflux. The data shown in Fig. 7 reveal that removal of external K⁺ has relatively little effect on K⁺ efflux. Since Na_i⁺ is so low in MAJ cells, it is possible that the ouabain-sensitive K⁺ efflux into K⁺-free medium occurs by the reversal of the pump (K_i⁺-Na_o⁺ exchange). This explanation is confirmed by the result that replacement of Na_o⁺ by TEA or choline reduces the ouabain-sensitive K⁺ efflux at 0 external K⁺ to zero (Fig. 8). The external Na⁺-activated, ouabain sensitive K⁺ efflux esti-

mated from the difference in K⁺ efflux into media containing either Na⁺ or choline (high Na⁺ and Na⁺-free media) provides a measure of K_i⁺-Na_o⁺ exchange. Using this measure it is possible to estimate the magnitude of K_i⁺-K_o⁺ exchange. With this approach, all of the ouabain-sensitive K⁺ efflux into high-Na⁺ medium could be accounted for either as K_i⁺-Na_o⁺ (reversal) or K_i⁺-K_o⁺ exchange provided that the stoichiometry of K_i⁺-K_o⁺ exchange is 3:2 (see Fig. 5).

The equilibrium point for the Na⁺/K⁺ pump in MAJ cells

The relatively large magnitude of the K_i⁺-Na_o⁺ exchange (reversal) flux in MAJ cells permitted a preliminary assessment of the equilibrium point of the Na⁺/K⁺ pump in MAJ red cells. For Na⁺ fluxes, the equilibrium point at which there is no net Na⁺ flux through the pump occurs when ouabain-sensitive Na⁺ efflux via Na_i⁺-K_o⁺ exchange equals ouabain-sensitive Na⁺ influx via the reversal mode (K_i⁺-Na_o⁺ exchange). As shown in Fig. 9, for MAJ cells containing 2.1 mmol/1 cell Na_i⁺, the Na⁺ fluxes through the normal and reversal modes are equal and opposite when K_o ~ 1.5 mM. Under these conditions, the concentration of ATP, ADP, and P_i in the cells are those listed in Table I. It will be interesting to observe the effect of varying Na_i⁺ on the value of K_o⁺ required to make the reversal and normal modes of ouabain-sensitive Na⁺ transport equal. Experiments to examine this point are planned.

Physiological consequences of increased density of Na⁺/K⁺ pumps in MAJ cells. It is impressive that MAJ cells appear to function normally despite the fact that they contain 10–20 times more Na⁺/K⁺ pumps than do normal red cells. This results from the autoregulation of the pump by Na_i⁺. As shown in Table I, Na_i⁺ in MAJ cells is about one-fourth the value found in normal human red cells. Since the flux through the normal mode of the pump increases steeply with Na_i⁺ in this concentration range (Fig. 1), the reduction in Na_i⁺ brings the flux through the normal mode when MAJ cells are incubated in a plasma-like medium (Na_o⁺ = 140 mM, K_o⁺ = 4 mM) to a value of 1.8 mmol/1 cell per h, similar to that observed in normal red cells containing 8–

9 mmol/l cell of internal Na^+ . In other words, the reduction in Na^+ causes the pump in MAJ cells to operate physiologically at a lower fraction of maximum capacity than is the case with normal red cells. Since the coupling of ATP consumption and glycolysis to the Na^+/K^+ pumps appears to be normal in MAJ cells (Fig. 11), the rate of glycolysis is also normal in MAJ cells incubated under physiological conditions.

Since MAJ cells maintain a steady state distribution of Na^+ and K^+ when the pump is operating at a normal rate, it follows that the net downhill or leakage fluxes of Na^+ and K^+ through the other monovalent cation transport pathways (Na^+/K^+ cotransport, KCl cotransport, $\text{NaCO}_3^-/\text{HCO}_3^-$ exchange, electrochemical diffusion, etc.) must also be within normal limits. This suggests that the membrane density of these pathways is not increased in MAJ cells. It has sometimes been proposed that some of the leakage of Na^+ and K^+ occurs through the pump itself, perhaps in a mode that is not inhibited by ouabain. The observations in MAJ cells do not support this proposal because the net movement of ions through the leakage pathway is within normal limits despite a Na^+/K^+ pump density that is more than 10 times normal.

What causes the increased membrane density of Na^+/K^+ pumps in MAJ cells? A clear answer to this question is not now possible. It has been shown that prolonged exposure of different cultured cells to ouabain induces an increase in the number of functional Na^+/K^+ pumps in the cell membrane (25). Since it has been proposed that endogenous inhibitors of the Na^+/K^+ pump might exist in human plasma (26) and cerebrospinal fluid (27), we investigated the hypothesis that a circulating inhibitor of active Na^+ and K^+ transport could be responsible of the increased membrane density of pumps in MAJ cells. The results of our experiments (not shown) do not support this possibility.

The number of pumps per cell or per unit membrane area in reticulocytes and in stem cells of the erythroid series is much higher than the number present in the mature human red cell (28). Red cell maturation seems to involve, among other important developments, the loss of active pump units from the membrane. Possibly, the process by which pumps are destroyed or inactivated is impaired in MAJ cells. If this is the case, the process must be quite specific for the Na^+/K^+ pump, because the membrane density of other Na^+ and K^+ transport proteins does not appear to be increased in MAJ cells.

Acknowledgments

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