Characterization of Two Independent Modes of Action of ATP on Human Erythrocyte Sugar Transport†

Amy L. Helgerson, Daniel N. Hebert, Shokofeh Naderi, and Anthony Carruthers*
Department of Biochemistry, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01605
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ABSTRACT: Intracellular ATP has been reported either to stimulate [Jacquez, J. A. (1983) *Biochim. Biophys. Acta 727*, 367-378] or to inhibit [Hebert, D. N., & Carruthers, A. (1986) *J. Biol. Chem. 261*, 10093-10099] human erythrocyte sugar transport. This current study provides a rational explanation for these divergent findings. Protein-mediated 3-*O*-methyl-α-D-glucopyranoside (3OMG) uptake by intact human red blood cells (lacking intracellular sugar) at ice temperature in isotonic KCl containing 2 mM $MgCl_2$, 2 mM EGTA, and 5 mM Tris-HCl, pH 7.4 (KCl medium), is characterized by a $K_{m(app)}$ of 0.4 ± 0.1 mM and a V_{max} of 114 ± 20 μmol L^{-1} min^{-1}. Lysis of red cells in 40 volumes of EGTA-containing hypotonic medium and resealing in 10 volumes of KCl medium increase the $K_{m(app)}$ and V_{max} for uptake to 7.1 ± 1.8 mM and 841 ± 191 μmol L^{-1} min^{-1}, respectively. Addition of ATP (4 mM) to the resealing medium restores Michaelis and velocity constants for zero-trans 3OMG uptake to 0.42 ± 0.11 mM and 110 ± 15 μmol L^{-1} min^{-1}, respectively. Addition of $CaCl_2$ to extracellular KCl medium (calculated $[Ca^{2+}]_o$ = 101 μM) reduces the V_{max} for zero-trans 3OMG uptake in intact cells and ATP-containing ghosts by 79 ± 4% and 61 ± 9%, respectively. Intracellular Ca^{2+} (15 μM) reduces the V_{max} for 3OMG uptake by ATP-containing ghosts by 38 ± 12%. In nominally ATP-free ghosts, extracellular (101 μM) and intracellular (11 μM) Ca^{2+} reduce the V_{max} for 3OMG uptake by 96 and 94%, respectively. Uptake is inhibited half-maximally in ATP-containing ghosts by 2.2 μM extracellular Ca^{2+} and by 2.4 μM intracellular Ca^{2+}. The effect of extracellular Ca^{2+} on uptake is reversible in intact cells and ghosts containing ATP but not in ATP-free ghosts. While a nonmetabolizable analogue of ATP (adenylyl imidodiphosphate) can substitute for ATP in reducing the $K_{m(app)}$ and V_{max} for uptake in Ca^{2+}-free ghosts, this ATP analogue cannot substitute for ATP in ATP-reversal of Ca^{2+} inhibition of sugar uptake. These results suggest two unrelated actions of ATP on erythrocyte sugar transport: (1) a kinase-independent reduction of $K_{m(app)}$ and V_{max} for uptake; (2) a hydrolysis-dependent reversal of Ca inhibition of uptake. These findings are discussed within the context of previous studies, and a hypothesis for ATP and Ca^{2+}-modulation of RBC sugar transport is proposed.

A number of contradictory reports describe the effects of intracellular ATP on human erythrocyte sugar transport (Table I). The effects range from an inhibitory action through no effect to a stimulation of transport. While these studies employed different methods of transport measurements and different methods of manipulation of intracellular ATP levels (e.g., pharmacologic, preparation of red cell ghosts, and the use of outdated blood), it remains difficult to rationalize these divergent findings. In this study, we present findings that can account for many of these apparent discrepancies.

We examined the effects of Ca^{2+}, ATP, and Ca^{2+} plus ATP on protein-mediated (cytochalasin B inhibitable) 3-*O*-methyl-α-D-glucopyranoside (3OMG)[1] uptake by human red cells and red cell ghosts at temperatures (0-4 °C) where reliable estimates of initial rates of transport may be obtained by using conventional stopping procedures. Our results demonstrate the following: (1) In the absence of Ca^{2+}, ATP reduces the $K_{m(app)}$ and V_{max} for 3OMG uptake by red cells by a kinase-independent reaction. (2) $Ca^{2+}{}_i$ and $Ca^{2+}{}_o$ reduce the V_{max} for 3OMG uptake by red cells and red cell ghosts resealed in the absence or presence of ATP. (3) Inhibition of uptake by Ca^{2+} is blunted by intracellular ATP. (4) ATP-free ghosts formed from Ca^{2+}-treated red cells retain inhibited uptake rates relative to ATP-free ghosts formed from Ca^{2+}-naive cells. (5) ATP_i reverses Ca inhibition of transport, but the nonhydrolyzable analogue of ATP adenylyl imidodiphosphate (AMPPNP) is unable to mimic ATP in this action.

We discuss these findings in the context of previous studies and propose an hypothesis that is consistent with many of the published data.

Materials and Methods

Materials. Cytochalasin B, 3-*O*-methyl-α-D-glucopyranoside, EGTA, and ATP were purchased from Sigma Chemicals. AMPPNP was purchased from Boehringer Mannheim Biochemicals. [^{14}C]-3OMG was purchased from New England Nuclear. Outdated whole human blood was obtained from the University of Massachusetts Medical Center Blood Bank.

Solutions. KCl medium consisted of 150 mM KCl, 2 mM $MgCl_2$, 2 mM EGTA, and 5 mM Tris-HCl, pH 7.4 (4 °C). Stopper solution consisted of KCl medium plus 1.5 mM KI, 1 μM $HgCl_2$, and 10 μM CCB at ice temperature. Lysis

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* Author to whom correspondence should be addressed.

[1] Abbreviations: 3OMG, 3-*O*-methyl-α-D-glucopyranoside; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; AMPPNP, adenylyl imidodiphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; CCB, cytochalasin B; RBC, red blood cell; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Sp, sphingomyelin; PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine.

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Table I: Effects of Various Cellular Manipulations on Sugar Transport in Human Erythrocytes[a]

manipulation	uptake[b] $K_{m(app)}$	uptake[b] V_{max}	exit[c] $K_{m(app)}$	exit[c] V_{max}	equilibrium exchange[d] $K_{m(app)}$	equilibrium exchange[d] V_{max}	ref
ghosts[e]							
	/[f]	/	normal[g]	normal	/	/	Challis et al. (1980)
	↑[h]	small ↑	↓[i]	normal	/	/	Taverna & Langdon (1973)
	↑	↑	/	/	/	/	Jung et al. (1971)
	↑	↑	↓	normal	/	/	Carruthers & Melchior (1983)
iodoacetamide	/	/	↓	↓	/	/	Carruthers (1989)
	↑	↑	↓	normal	/	/	Hebert & Carruthers (1986)
cytosol$_i$	normal	normal	normal	normal	/	/	Carruthers & Melchior (1983)
ATP_i	normal	normal	normal	normal	/	/	Hebert & Carruthers (1986)
$ATP_i + Ca^{2+}{}_i$	↑	↓	normal	↓	/	/	Hebert & Carruthers (1986)
170 μM ATP_i	/	/	↑	normal	↓	normal	Jensen & Brahm (1987)
2 mM ATP_i	/	/	↑	normal	normal	normal	Jensen & Brahm (1987)
intact red cells[j]							
A23187, 0 Ca^{2+}	normal	normal	/	/	/	/	Jacquez (1983)
A23187 + Ca^{2+}	normal	↓	/	/	/	/	Jacquez (1983)
A23187 + Ca^{2+} → 0 Ca^{2+}[k]	normal	↓	/	/	/	/	Jacquez (1983)
A23187 + Ca^{2+} → 0 Ca^{2+} + glucose[l]	normal	normal	/	/	/	/	Jacquez (1983)
A23187 + Ca^{2+} → ghosts[m]	normal	↓	/	/	/	/	Jacquez (1983)
A23187 + Ca → ghosts + ATP[n]	normal	normal	/	/	/	/	Jacquez (1983)
iodoacetamide	normal	↓	/	/	/	/	Jacquez (1983)
outdated blood[o]							
	normal	normal	/	/	/	/	Lacko et al. (1972)
	/	/	/	normal	/	/	Sen & Widdas (1962), Carruthers & Melchior (1983)[p]
	/	/	↓	normal	/	/	Carruthers & Melchior (1983)
	/	/	/	/	↑	↓	Weiser et al. (1983)
0 °C	↑	normal	normal	↓	↑	normal	Wheeler (1986)

[a] All experiments were at 20–25 °C unless stated otherwise. [b] Zero-trans uptake (uptake by sugar-free cells). [c] Zero-trans exit (sugar efflux into sugar-free medium). [d] Equilibrium exchange (radiolabeled sugar exchange rates in cells where $[sugar]_i = [sugar]_o$). [e] Parameters measured in ghosts are compared to those reported for intact cells. [f] Slash indicates these parameters were not measured. [g] Normal implies no change. [h] ↑ denotes an increase. [i] ↓ denotes a decrease. [j] In these studies, Jacquez (1983) employed outdated blood, and the changes reported are relative to untreated, outdated blood. [k] A23187 + Ca-treated cells were subsequently incubated in Ca-free medium. [l] A23187 + Ca-treated cells were subsequently incubated in Ca-free medium containing glucose. [m] A23187 + Ca-treated cells were subsequently lysed to form resealed ghosts lacking ATP and Ca. [n] A23187 + Ca-treated cells were subsequently lysed to form resealed ghosts containing ATP but lacking Ca. [o] Here parameters are compared to those reported for freshly drawn red blood cells. [p] Comparison of exit times for 60 mM glucose exit from fresh (Sen & Widdas, 1962) and outdated (Carruthers & Melchior, 1983) blood using turbidimetry.

medium consisted of 10 mM Tris-HCl and 2 mM EGTA, pH 7.4. Phosphate medium contained 150 mM KCl and 0.5 mM KH_2PO_4, pH 7.2. Lysis medium for ghosts subsequently containing phosphate medium consisted of 3 mM $MgSO_4$ and 3.8 mM acetic acid adjusted to pH 6.2 with Tris base. In experiments where ATP, $CaCl_2$, or ATP plus $CaCl_2$ were added to the various media, the pH was always readjusted to 7.4 or 7.2 (for KCl and phosphate media, respectively) using Tris base.

Red Cells and Red Cell Ghost Preparation. Washed red cells and resealed red cell ghosts were prepared as described by Carruthers and Melchior (1983). Briefly, red cells were obtained from outdated blood by three centrifugation/wash cycles in KCl medium. Between each centrifugation/wash step, the buffy coat containing white cells and platelets was carefully aspirated. Red cells were incubated in 100 volumes of KCl medium (1 h at 24 °C) to deplete intracellular glucose levels and then collected by centrifugation and stored on ice. Red cell ghosts were prepared by dispersing 1 volume of cells in 40 volumes of ice-cold lysis medium. Following 10 min on ice, the suspension was centrifuged, the supernatant containing cellular contents was aspirated, and the red cell membrane pellet was gently resuspended in 10 volumes of KCl medium. The suspension was then warmed to 37 °C and incubated for 40 min at this temperature. The resulting resealed ghosts were then collected by centrifugation, suspended in KCl medium, and placed on ice until use. When ghosts were formed containing ATP and Ca^{2+}, ATP and $CaCl_2$ were included in the KCl medium solution (pH adjusted to 7.4) used to reseal the ghosts. In some experiments, we employed the media (phosphate medium and lysis medium) described in Jensen and Brahm (1987) and above.

Zero-Trans 3-O-Methylglucose Uptake Measurements. Sugar-free red cells or ghosts (at ice temperature) were exposed to 10 volumes of KCl medium (ice temperature) containing variable [3OMG] and [^{14}C]3OMG. Uptake was permitted to proceed for a fixed period of time and then 50 volumes (relative to cell volume) of stopper solution was added to the cell suspension. The cells were sedimented by centrifugation, washed once more in stopper, collected by centrifugation, and extracted in 1 mL of 2% perchloric acid. Control experiments indicated that an additional two wash/centrifugation cycles at 4 °C in stopper did not significantly deplete the amount of radioactivity associated with the cells. The acid extract was centrifuged, and duplicate samples of the clear supernatant were counted. Zero-time uptake points were obtained by addition of stopper to cells before medium containing sugar and radiolabel, and then the cells were immediately processed and extracted. Radioactivity associated with cells at zero time was subtracted from the activity associated with cells following the uptake period. All uptakes were normalized to equilibrium uptake where cells were exposed to sugar medium at 37 °C for 0.5–2 h prior to addition of stopper.

Uptake was calculated in the following manner, defining the following: C_Z, radioactivity associated with cells at zero time; C_T, radioactivity associated with cells at t minutes; C_E, radioactivity associated with cells at equilibrium.

$$\text{uptake, } v = \frac{[3OMG](C_T - C_Z)}{(C_E - C_Z)t}$$

Estimates of rates of non-protein-mediated 3OMG uptake were made in parallel in each experiment in which cells were preincubated with 10 μM CCB [a transport inhibitor, $K_{i(app)}$ for noncompetitive inhibition of uptake is 129 ± 14 nM]. CCB (10 μM) was also included in the uptake medium during these uptake measurements. In the studies reported here, 3OMG uptake in cells and ghosts was more than 97% inhibited by 10 μM CCB.

In order to assess whether initial rates of 3OMG transport can be obtained accurately at ice temperature, 3OMG uptake by red cells at ice temperature at varying $[3OMG]_o$ (0.1–5 mM) was measured at 10, 20, and 30 s. Uptake is a linear function of time during this period at each $[3OMG]_o$ tested. As $[3OMG]_o$ is increased, so the half-time for uptake increases. Our results show that 3OMG uptake by intact cells is characterized by a V_{max} of 0.114 mmol L^{-1} min^{-1} and a $K_{m(app)}$ of 0.4 mM (see Table II below). This corresponds to a maximum rate constant for uptake of 0.285 min^{-1} [V_{max} $K_{m(app)}$] or a half-time for uptake at limitingly low [3OMG] of 146 s. In the studies described below, we employed 10–30-s intervals for uptake determination in order to limit the 3OMG space of the cells to 14% or lower. However, it is possible that the time course for uptake deviates from linearity at very low [3OMG] and $K_{m(app)}$ for uptake is overestimated in some experiments. We assessed this possibility by modeling the time course of 3OMG uptake assuming $K_{m(app)}$ and V_{max} values of 0.4 mM and 114 μmol L^{-1} min^{-1}, respectively, and additionally assuming that uptake follows a monoexponential time course where the rate constant for uptake, k is given by theoretical, Michaelis–Menten initial rate/[3OMG]. We then calculated the expected 3OMG space of the cells at 30 s and from this derived an estimate of the initial rate of uptake. Using these calculations, we obtained $K_{m(app)}$ and V_{max} for uptake by Hanes–Woolf analysis of 0.429 mM and 114 μmol L^{-1} min^{-1}, respectively. These considerations, while constrained by the arbitrary assumption of exponential sugar uptake, suggest that the use of a 30-s sampling period results in overestimation of the $K_{m(app)}$ for sugar uptake by only 7% without affecting the reliability of estimates of V_{max}.

Equilibrium exchange 3OMG exit at 4 °C ($[3OMG]_i$ = $[3OMG]_o$) was measured as in Helgerson and Carruthers (1989).

ATP Assays. Red cell ATP content was determined as described by Hebert and Carruthers (1986).

Lipid Analyses. Intact red cell and red cell ghost membrane lipids were extracted by a modification of the Bligh and Dyer technique (Bligh & Dyer, 1959). Briefly, 1 volume of RBCs or ghosts was homogenized in 1 volume of butanol/water (1:1 by volume). The homogenate was centrifuged lightly and the upper phase collected. The lower phase was extracted 3 more times in butanol/water and the upper phases combined. The upper butanol phase was washed 3 times with 4 mL of water, and then the butanol was removed by rotary evaporation in a round-bottomed flask. The dried, lipid film was dissolved in chloroform/methanol (2:1 by volume) and placed in a conical vial. The organic sovents were removed under N_2, and then the lipid was redissolved in a small, but known amount of chloroform/methanol. The vial was flushed with N_2, capped, and stored at −70 °C.

Phospholipids were separated according to lipid class (lipid headgroup composition) by two-dimensional thin-layer chromatography using silica plates (Prekotes Adsorbosil, Alltech) and solvent systems of chloroform/methanol/ammonium hydroxide (78:30:6 by volume) in the first dimension and chloroform/acetone/methanol/acetic acid/water (36:48:12:12:6 by volume) in the second dimension. The silica plates were prewashed in both dimensions with drying after each wash. Immediately prior to use, the plates were activated by heating to 110 °C for 1 h. Lipid extract (5 μL) was spotted onto the plate with internal authentic standards, or standards were run on parallel plates. Following development, lipid spots were detected by exposing the plates to I_2 and circled. The spots were scraped off the plates, and their phosphorus content was assayed by using the Bartlett procedure (Bartlett, 1959). Total applied phosphorus was determined by assaying 5 μL of lipid extract.

Nonpolar lipids were separated by one-dimensional thin-layer chromatography using silica gel G plates (Alltech) and successive solvent systems of diethyl ether/benzene/ethanol/acetic acid (40:50:2:0.2 by volume) followed by diethyl ether/hexane (6:94 by volume). Following development, the plates were sprayed with saturated $K_2Cr_2O_7$ in 80% H_2SO_4 and lipid spots detected by charring. Authentic standards (cholesterol, cholesterol ester, and diacylglycerol) were run on parallel plates. Lipids were quantitated (relatively) by reflectance scanning densitometry, and cholesterol and cholesterol esters were quantitated directly by the method of Gamble et al. (1978).

Calculation of Ionized Calcium Levels. The log apparent dissociation constants (K) for Ca and Mg binding to EGTA and ATP at a given pH were calculated by means of a short computer program using the log equilibrium constants cited by Sillen and Martell (1964) for the interaction of H^+, Ca^{2+}, Mg^{2+}, and, when available, K^+ with EGTA, PO_4^{3-}, and ATP at 20 °C. The apparent dissociation constants calculated for EGTA at pH 7.4 are as follows (−log): K_{Ca} = 7.3; K_{Mg} = 2.05. For PO_4^{3-} at pH 7.2, they are K_{Ca} = 2.534 and K_{Mg} = 2.69. For ATP at pH 7.4, they are K_{Ca} = 3.84 and K_{Mg} = 3.98 and 3.82 and 3.89, respectively, at pH 7.2. These values were inserted into a second program with total Ca, Mg, K, ATP, and EGTA or PO_4^{3-} levels. This program computes ionized cation levels by an iterative procedure until the results change by less than 1 part in 10^{15}. The results of these calculations require assumptions with respect to the purity of ligand and chelator solutions. Thus, we also refer under Results to Ca:EGTA ratios at fixed Mg, EGTA, and ATP levels.

Measurement of the Ca^{2+} Content of Phosphate Medium. The ionized Ca content of phosphate medium (±4 mM ATP) was determined by using an MI-600 Ca electrode (Microelectrodes Inc.) calibrated against KCl medium (pH 7.2) containing 0.5, 1, 1.5, 2, and 2.1 mM $CaCl_2$ (calculated $[Ca^{2+}]$: 0.04622, 0.13917, 0.4185, 16.67, and 102.72 μM, respectively; K_{Ca} = 6.906 and K_{Mg} = 1.809 at pH 7.2). The resulting calibration (millivolts versus log [Ca]) yielded a slope of 26.87 mV per decade and a y intercept of 119.48 mV (R^2 = 0.984). Phosphate media ± 4 mM ATP gave readings of −14 and −46 mV, respectively. The extrapolated ionized Ca contents of phosphate medium with and without 4 mM ATP are thus 0.69 and 10.77 μM, respectively. KCl medium (pH 7.4) lacking exogenous Ca and calibrated against the above standards at pH 7.4 contained an estimated $[Ca^{2+}]$ of 0.46 nM which extrapolates to a total [Ca] of ≈15 μM.

Calculation of Michaelis and Velocity Parameters. [3OMG] vs uptake data were linearized in the form [3OMG]/uptake versus [3OMG] to obtain estimates of V_{max} (1/slope) and $K_{m(app)}$ ($-x$ intercept). These values were then inserted into a nonlinear regression program to obtain $K_{m(app)}$ and V_{max} parameters which provide the best fit (residuals are

Table II: Effects of $[ATP]_i$ and $[Ca^{2+}]$ on Zero-Trans 3-*O*-Methylglucose Uptake in Red Cells and Red Cell Ghosts[a]

$[ATP]_i$ (mM)	$[Ca^{2+}]_o$ (μM)[b]	$[Ca^{2+}]_i$ (μM)[c]	$K_{m(app)}$ (mM)	V_{max} (μmol L^{-1} min^{-1})	n[d]
			Red Cells		
1 ± 0.1	0	?	0.4 ± 0.1 (0.12–0.9)[e]	114 ± 20 (74–190)	6
0.8 ± 0.1	101	?	0.36 ± 0.03 (0.25–0.41)	23 ± 1*[f] (19–27)	4
			Red Cell Ghosts		
4	0	0	0.42 ± 0.11 (0.19–0.8)	110 ± 15 (70–151)	5
4	101	0	0.28 ± 0.04 (0.17–0.36)	43 ± 5* (38–49)	4
4	0	15	0.32 ± 0.05 (0.21–0.45)	68 ± 6* (46–77)	4
0	0	0	7.1 ± 1.8* (2.3–13)	841 ± 191* (204–1107)	5
0	101	0	2.9 ± 0.4* (1.7–3.8)	36 ± 7* (26–48)	4
0	0	11	2.7 ± 0.7* (1.9–4.3)	52 ± 5* (42–61)	4

[a] Results are shown as mean ± SE. [b] Ca:EGTA ratio = 2.1:2 at 2 mM $MgCl_2$, pH 7.4. [c] Ca:EGTA ratio = 2.2:2 at 2 mM $MgCl_2$ and 4 mM ATP, pH 7.4, and 2:2 at 2 mM $MgCl_2$ and 0 ATP, pH 7.4. [d] n is the number of separate experiments. [e] The range of measured values is shown in parentheses. [f] Asterisks indicate that the results are significantly different from control $K_{m(app)}$ and V_{max} values (red blood cell, 0 Ca^{2+}) at the $p < 0.05$ level (two-tailed t-test).

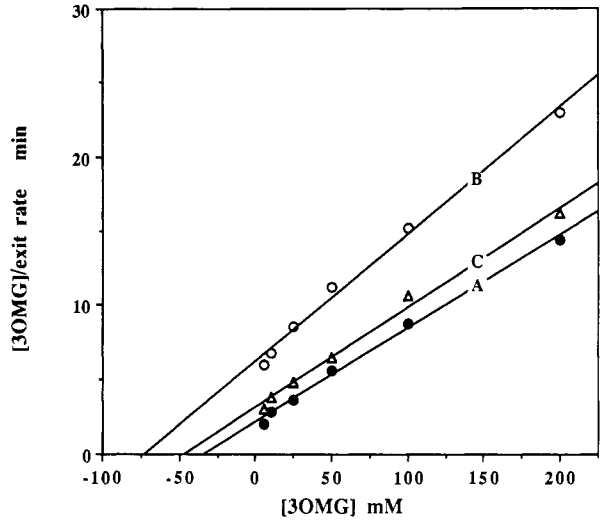

FIGURE 1: Effects of red cell lysis followed by resealing on equilibrium exchange 3OMG exit from red cells at ice temperature. Ordinate: [3OMG]/rate of 3OMG exchange in minutes. Abscissa: $[3OMG]_i$ and $[3OMG]_o$ in millimolar. Data points were obtained in duplicate. The lines drawn through the points were calculated by the method of least squares. The slope of these lines corresponds to $1/V_{max}$, and the x intercept is $-K_{m(app)}$. Line A (intact red cells), $K_{m(app)}$ = 33.4 mM and V_{max} = 15.9 mmol L^{-1} min^{-1}; line B (red cell ghosts), $K_{m(app)}$ = 72.4 mM and V_{max} = 11.7 mmol l^{-1} min^{-1}; line C (ghosts + 4 mM ATP_i), $K_{m(app)}$ = 45.4 mM and V_{max} = 15.2 mmol l^{-1} min^{-1}.

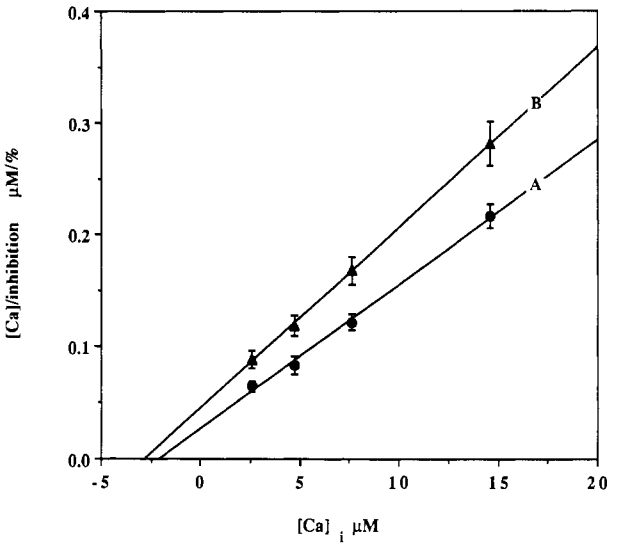

FIGURE 2: Inhibition of zero-trans 3OMG uptake in ghosts by intracellular Ca^{2+} in the presence of 4 mM ATP_i. Ordinate: calculated $[Ca^{2+}]_i$/percent inhibition of uptake (micromolar/percent). Abscissa: calculated $[Ca^{2+}]_i$ in micromolar. Curve A represents uptake measurements made at 0.2 mM 3OMG and curve B uptake measurements at 5 mM 3OMG. The data points show the mean ± SD of four separate determinations. The lines through the points were calculated by the method of least squares. The slope of each line = 1/maximum inhibition, and the x intercept = $-K_{i(app)}$. The calculated constants are as follows: (A) $K_{i(app)}$ = 2.0 μM Ca^{2+}, maximum inhibition (I_m) = 77.4%; (B) $K_{i(app)}$ = 2.8 μM Ca^{2+}, I_m = 62%. The ratios of exogenous $CaCl_2$ to EGTA employed to calculate the various Ca^{2+}_i levels (at 2 mM EGTA, 2 mM $MgCl_2$, and 4 mM ATP, pH 7.4) were 0:2, 2:2, 2.05:2, 2.1:2, and 2.2:2.

at a minimum) of the experimental data to the Michaelis–Menten equation (Duggleby, 1981). When $K_{m(app)}$ and V_{max} parameters are shown as mean ± SE, the SE does not refer to the standard error of the estimated nonlinear regression best fit but rather to the standard error of the mean of parameters calculated in more than three separate experiments.

Results

In the experiments described below, intact cells were exposed to KCl or phosphate medium containing variable $[Ca^{2+}]$ for 30 min at 24 °C prior to transport determinations at ice temperature. Ghosts were resealed in medium containing variable [ATP] and $[Ca^{2+}]$ for 40 min at 37 °C and then incubated for 30 min at 24 °C in medium containing variable $[Ca^{2+}]$ prior to transport determinations at ice temperature. Control experiments indicate that effects of Ca^{2+}_o and Ca^{2+}_i on red cell sugar transport are fully expressed by this time (not shown).

Table II summarizes the results of experiments from six separate batches of outdated human blood in which the [3OMG] dependence of 3OMG uptake at ice temperature was measured in intact cells and resealed ghosts. Uptake in red cells was measured in the absence and presence of 101 μM extracellular Ca^{2+}. Uptake by ghosts containing and lacking exogenous intracellular ATP (4 mM) and/or Ca^{2+} (11–15 μM) was measured in the presence or absence of extracellular Ca^{2+}. Preparation of ATP-free ghosts from red cells increases the $K_{m(app)}$ and V_{max} for 3OMG uptake. This effect is reversed by addition of ATP to the resealing medium. Inclusion of $CaCl_2$ in the ATP resealing medium further reduces the V_{max} for 3OMG uptake. External Ca reduces the V_{max} for uptake by intact cells and ghosts containing ATP.

$K_{m(app)}$ and V_{max} for equilibrium exchange of 3OMG exit from red cells are increased and decreased, respectively, in red cell ghosts relative to intact cells (Figure 1, EGTA present). These effect are partly restored by resealing ghosts in the presence of ATP (2 mM).

Intracellular Ca^{2+}. The $[Ca^{2+}]_i$ dependence of the inhibition of V_{max} for 3OMG uptake by intracellular Ca^{2+} is shown at saturating and subsaturating $[3OMG]_o$ in Figure 2. In these

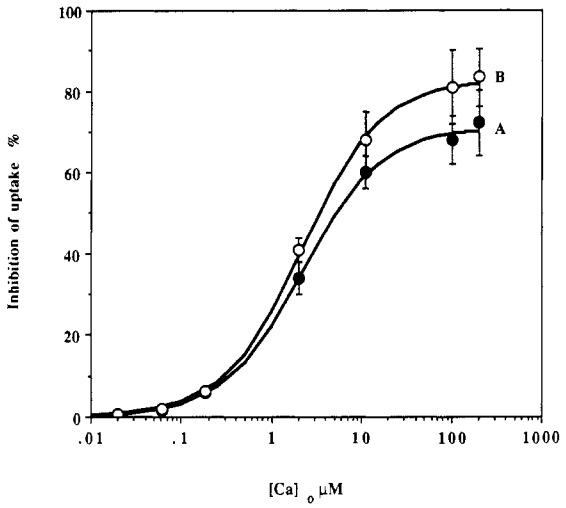

FIGURE 3: Inhibition of 3OMG uptake in red cells by Ca^{2+}_o. Ordinate: percent inhibition of 3OMG uptake. Abscissa: external $[Ca^{2+}]$ in micromolar (shown as log scale). Uptake was measured at 0.2 (A) and 5 (B) mM 3OMG. The data points represent the mean ± SD of four separate measurements. The curves drawn through the points were calculated by nonlinear regression and have the following constants: (A) $K_{i(app)}$ = 2.19 μM Ca^{2+}, I_m = 71%; (B) $K_{i(app)}$ = 2.21 μM Ca^{2+}, I_m = 82.5%. The exogenous $CaCl_2$:EGTA ratios employed to calculate the Ca^{2+}_o levels at 2 mM EGTA and 2 mM $MgCl_2$, pH 7.4, were 0:2, 0.5:2, 1:2, 1.5:2, 1.95:2, 2:2, 2.1:2, and 2.2:2.

experiments, the resealing medium contained 4 mM ATP. At both $[3OMG]_o$, uptake is half-maximally inhibited at ≈2.4 μM Ca^{2+}.

Extracellular Ca^{2+}. Table II shows that extracellular Ca^{2+} inhibits 3OMG uptake by reducing the V_{max} for sugar entry. Under these conditions, intracellular [ATP] is reduced by 20% by the addition of extracellular Ca [$[ATP]_i$ = 1.0 ± 0.1 mM (0 Ca) and 0.8 ± 0.1 mM (101 μM ionized Ca)]. Figure 3 shows the $[Ca^{2+}]_o$ dependence of this effect on transport at saturating and subsaturating $[3OMG]_o$. Uptake is half-maximally inhibited at ≈2.2 μM Ca^{2+}_o.

Inhibition of 3OMG uptake by Ca^{2+}_o does not result from increased $[Ca^{2+}]_i$ and seems not to require ATP_i. Tables II and III show that Ca_o inhibits 3OMG uptake in red cell ghosts containing 2 mM EGTA and 0 $CaCl_2$ ± 4 mM ATP although uptake inhibition is somewhat blunted in the presence of ATP_i. Inhibition by Ca^{2+}_i plus Ca^{2+}_o is additive (Table III). Inhibition by Ca^{2+}_i does not require the simultaneous presence of exogenous ATP_i (Table III). Inhibition by Ca^{2+}_o in Ca^{2+}-free, ATP-free ghosts is unaffected by the presence of 2 mM $AMPPNP_i$ (a nonmetabolizable analogue of ATP, Table III).

It is important to remember in examination of Table III (and Tables IV and V, see below) that in the absence of Ca^{2+} and ATP, uptake by ghosts at 0.2 mM 3OMG is reduced and uptake at 5 mM 3OMG is increased. ATP acts to reduce the $K_{m(app)}$ and V_{max} for uptake in ghosts and thus stimulates uptake at subsaturating (≤1 mM) [3OMG] but inhibits uptake at saturating [3OMG].

Reversibility. Table IV summarizes experiments that examine the reversibility of Ca^{2+} effects on transport. Ca^{2+} inhibition of RBC 3OMG uptake is largely reversed by incubation of Ca^{2+}-treated cells in Ca^{2+}-free medium for 30 min. If Ca^{2+}-treated and untreated cells are lysed and then resealed in ATP-free medium, ghosts from Ca^{2+}-treated cells display lower uptake rates than do ghosts from untreated cells. If membranes from these cells are resealed in the presence of ATP, uptake in ghosts from Ca^{2+}-treated and untreated cells is indistinguishable. These data suggest that the reversal of

Table III: Effects of Ca^{2+} on 3OMG Uptake in Red Cell Ghosts[a]

condition	3OMG uptake (μmol L^{-1} min^{-1}) for [3OMG] (mM) of uptake determination: 0.2	5
cells	24.0 ± 1.2	112 ± 5
cells + 100 μM Ca^{2+}_o[b]	8.1 ± 1.3[e]	26 ± 3[e]
ghosts + 0 ATP_i[c]	8.1 ± 0.5[e]	236 ± 13[f]
+11 μM Ca^{2+}_i[d]	3.2 ± 0.1[e]	57.5 ± 6.0[e]
+100 μM Ca^{2+}_o[b]	3.3 ± 0.9[e]	43 ± 2.8[e]
+Ca^{2+}_i + Ca^{2+}_o	3.4 ± 0.2[e]	26.5 ± 1.8[g]
ghosts + 4 mM ATP_i[c]	24.5 ± 0.4	146 ± 6[f]
+15 μM Ca^{2+}_i[d]	15.6 ± 0.4[e]	87 ± 1.4[e]
+100 μM Ca^{2+}_o[b]	14.8 ± 0.2[e]	88.5 ± 1.8[e]
+Ca^{2+}_i + Ca^{2+}_o	13.2 ± 0.6[e]	68.1 ± 0.7[g]
ghosts + 2 mM AMPPNP	23.1 ± 0.7	
+100 μM Ca^{2+}_o[b]	9.6 ± 0.4[e]	

[a] The number of separate duplicate determinations for uptake at 0.2 mM 3OMG and for uptake at 5 mM 3OMG was 4 in each case. [b] Ca:EGTA ratio = 2.1:2 at 2 mM $MgCl_2$, pH 7.4. [c] Ghosts were resealed in the absence or presence of 4 mM exogenous ATP. [d] Ca:EGTA ratio = 2.2:2 at 2 mM $MgCl_2$/4 mM ATP, pH 7.4, and 2:2 at 2 mM $MgCl_2$/0 ATP, pH 7.4. [e] Significantly less than control values at the $p < 0.01$ level (one-tailed t-test). [f] Significantly greater than control values at the $p < 0.01$ level (one-tailed t-test). [g] The inhibition by Ca^{2+}_i plus Ca^{2+}_o is significantly greater than that by Ca^{2+}_i or Ca^{2+}_o alone.

Table IV: Reversibility of Ca^{2+} Action on 3OMG Uptake[a]

origin[b]	$[Ca^{2+}]_o$ (μM)	$[ATP]_i$[c] (mM)	$[AMPPNP]_i$ (mM)	3OMG uptake at 1 mM (μmol L^{-1} min^{-1})
		Cells		
A	0	1.0 ± 0.1	0	53.0 ± 6.2
B	100[d]	0.8 ± 0.1	0	24.6 ± 4.3[e]
B	100 → 0	?	0	47.9 ± 4.0
		Ghosts		
A	0	0	0	29.8 ± 1.0[e]
B	0	0	0	13.7 ± 0.1[e,f]
A	0	4	0	61.5 ± 4.6
B	0	4	0	51.7 ± 4.1
A	0	0	4	55.5 ± 3.2
B	0	0	4	18.1 ± 1.0[e,f]

[a] The number of separate measurements made was four. Results are shown as mean ± SE. [b] Origin refers to original treatment of cells. "A" cells were originally Ca naive. "B" cells were initially exposed to 100 μM Ca^{2+} for 30 min at 24 °C. $[Ca^{2+}]_o$ refers to the concentration of Ca^{2+} present during uptake measurements. [c] For intact cells, $[ATP]_i$ refers to the measured ATP content (millimoles per killigram of cell water) of cells and for ghosts refers to the initial [ATP] of the resealing medium. [d] Ca:EGTA ratio = 2.1:2 at 2 mM $MgCl_2$, pH 7.4. [e] Indicates that uptake is significantly lower than uptake measured in Ca^{2+}-naive intact red cells ($p < 0.01$, one-tailed t-test). [f] Indicates that uptake by B cell ghosts is significantly less ($p < 0.01$, one-tailed t-test) than uptake by A cell ghosts resealed under identical conditions.

Ca^{2+} effects on transport requires intracellular ATP. AMPPNP (a nonhydrolyzable ATP analogue) cannot substitute for ATP in this reversal of Ca^{2+} inhibition of transport (Table IV). Modulation of uptake in the absence of Ca^{2+} by intracellular ATP seems not to require ATP hydrolysis as the nonhydrolyzable analogue of ATP (AMPPNP) can substitute for ATP in this action (Table IV).

Effects of Different Media. Sugar uptake by intact cells, ghosts, and ghosts resealed in the presence of ATP (4 mM) is modified if EGTA and $MgCl_2$ containing medium is replaced with 150 mM KCl and 0.5 mM PO_4^{3-}, pH 7.2 (a medium used in many of the previous studies summarized in Table I). With intact cells, uptake experiments were made in both phosphate and EGTA medium. With ghosts, the membranes were resealed in the presence of EGTA or phosphate media, and then all uptakes were measured in EGTA

Table V

condition	3OMG uptake[a] (μmol L^{-1} min^{-1}) measured at 0.2 mM 3OMG	5 mM 3OMG
cells		
KCl medium (pH 7.4)	25.5 ± 0.4	116 ± 6
KCl medium (pH 7.2)	26.8 ± 0.5	124 ± 7
PO_4^{3-} medium (pH 7.2)	16.1 ± 0.2[c]	85 ± 8[c]
ghosts[b] resealed in KCl medium		
pH 7.2	11.3 ± 0.8[c]	201 ± 16[c]
pH 7.4	10.7 ± 0.9[c]	207 ± 14[c]
pH 7.4 ± 4 mM ATP	22.1 ± 2.2	113 ± 5
ghosts resealed in PO_4^{3-} medium		
pH 7.2	6 ± 1[c]	63 ± 7[c]
pH 7.2 + 4 mM ATP	22.7 ± 4.3	144 ± 19

[a] Results are shown as mean ± SE. The number of separate determinations made for uptake at 0.2 and 5 mM 3OMG was 4 in each instance. [b] In these experiments, ghosts were resealed in the medium described, and uptake measurements were made in KCl medium (pH 7.4, $[Ca^{2+}] < 1$ nM). [c] Indicates that uptake is significantly different ($p < 0.01$, two-tailed t-test) from control uptake (uptake from KCl medium by intact cells).

medium. Sugar uptake by intact cells in phosphate medium is reduced compared to that observed in EGTA medium (Table V). This does not result from the reduced pH of phosphate medium. Uptake in ghosts containing phosphate medium is reduced relative to ghosts containing EGTA medium (Table V). This does not result from the reduced pH of phosphate medium. Addition of ATP to phosphate medium during ghost resealing reverses the inhibition of transport observed in ghosts lacking ATP (Table V). Assuming a contaminating Ca_{total} level of 12.5 μM in phosphate medium, the calculated ionized Ca levels in the absence and presence of 4 mM ATP would be 10.6 and 0.4 μM, respectively. The measured levels were 10.8 and 0.7 μM, respectively. It is possible, therefore, that phosphate medium inhibits 3OMG uptake due to the presence of Ca^{2+} and that the reversal of this effect in ghosts by ATP results from (1) Ca^{2+} sequestration by ATP and (2) the observed ATP reversal of Ca-mediated transport inhibition. Assuming Mg·ATP mediates ATP reversal of Ca^{2+} inhibition of transport, this latter effect would require the simultaneous presence of contaminating Mg.

Red Cell Lipid Content. Table VI summarizes lipid analyses of RBCs and ghosts exposed to varying $[Ca^{2+}]_{i,o}$ and $[ATP]_i$. Other than Ca^{2+}_i-induced increases in the phosphatidic acid content of ATP-containing ghosts, no systematic effects of Ca^{2+} and ATP treatment on red cell membrane lipid composition were observed.

Discussion

This study was performed in an attempt to reconcile dissonant findings resulting from a number of studies investigating the relationship between human erythrocyte sugar transport rates and intracellular [ATP]. These studies fall into three main groups: (1) studies employing the method of red cell ghost preparation to dilute intracellular contents; (2) studies employing metabolic perturbations via pharmacologic manipulations of red cells; (3) the use of outdated blood. Each approach presents unique problems in data interpretation. The use of red cell ghosts, while avoiding the use of pharmacologic agents and presenting a means of controlling intracellular solute conditions, could also result in the loss of intracellular materials other than ATP that interact with the red cell hexose transfer system. The preparation of ghosts in the absence of Ca-chelating agents results in the loss of bilayer lipid asymmetry (Schlegel et al., 1985). The pharmacologic approach, while employing intact cells, could also result in a multiplicity of pharmacologic perturbations of both the transport system and intracellular metabolites. The use of outdated blood, while avoiding red cell lysis and pharmacologic manipulations, introduces variables such as cellular aging and loss of bilayer lipid asymmetry (Alam, 1985).

Table I represents an effort to summarize the results of studies employing the above approaches to this problem. It is difficult to form a consistent picture from these data. While clusters of studies tend to show some agreement, similar studies from different laboratories can show a surprising degree of inconsistency. For example, while Weiser et al. (1983) report that $K_{m(app)}$ for equilibrium exchange transport is increased and the V_{max} is decreased in outdated blood at 20 °C, Jensen and Brahm (1987) report that the $K_{m(app)}$ for exchange is reduced and the V_{max} is unaffected in outdated blood at 25 °C. Our studies show that intracellular ATP acts to reduce the $K_{m(app)}$ and to increase the V_{max} for equilibrium exchange 3OMG exit at ice temperature. Jacquez (1983) reports that iodoacetamide results in a reversible inhibition of glucose uptake by red cells. We have found that iodoacetamide irreversibly inhibits efflux in red cell ghosts lacking ATP, suggesting a direct inhibitory action on the transport system (Carruthers, 1989).

Table VI: Analysis of the Lipid Content (Percent of Total Measured Lipid) of Red Cell Membranes[a]

	RBC (A)	RBC (B)	B[b] → 0 Ca_o	A[c] ghosts			B[d] ghosts		
ATP_i[e]	+	+	+	−	+	+	−	+	+
Ca_o	−	+	±	−	−	−	−	−	−
Ca_i	−	−	−	−	−	+	−	−	+
total lipid[f]									
phospholipid	47.0	47.7	47.8	52.2	50.3	49.1	50.0	46.6	47.5
cholesterol	45.8	44.4	45.6	40.8	43.3	44.9	42.2	44.5	43.9
DAG	7.2	7.9	6.6	7.0	6.4	6.0	7.8	8.9	8.6
phospholipid species[g]									
PC	14.1	14.0	13.8	14.5	15.1	15.2	15.0	15.9	14.2
PE	13.1	13.9	13.0	15.1	12.8	12.3	12.2	11.7	12.3
Sp	12.2	12.1	13.2	14.2	13.4	13.0	13.8	11.1	12.5
PA	1.2	1.2	1.1	1.3	1.2	1.9	1.1	1.3	2.6
PS/PI[h]	5.0	4.9	5.3	5.3	5.5	4.7	5.4	5.0	4.3
LPC	1.4	1.6	1.4	1.8	2.3	2.0	2.5	1.6	1.6

[a] Lipid species (acyl chain composition) were not identified. The results are shown as the mean of two separate determinations. [b] B cells (previously incubated in Ca medium) were incubated in Ca-free medium for 30 min. [c] Ghosts were made from A cells (Ca-naive). [d] Ghosts were made from B cells (Ca-exposed). [e] The ATP content of the ghost resealing medium was 2 mM. The ATP content of red cells was not determined. [f] Total lipid content includes phospholipids, cholesterol, and diacylglycerol (DAG). [g] The phospholipid species are as follows: PC, phosphatidylcholine; PE, phosphatidylethanolamine; Sp, sphingomyelin; PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphoatidylcholines. [h] PS and PI comigrate as a single spot using this chromatography system.

Jensen and Brahm (1987) report that the formation of red cell ghosts results in an increased $K_{m(app)}$ for exit relative to that in intact cells, an effect partly reversed by resealing with ATP. Challis et al. (1980) report no effect of red cell lysis and resealing on the $K_{m(app)}$ for exit. Taverna and Longdon (1973), Carruthers and Melchior (1983), and Hebert and Carruthers (1986) report lysis and resealing of red cells are without effect on the V_{max} for glucose exit but reduce the $K_{m(app)}$ for exit, an effect reversed by resealing ghosts with cytosol (Carruthers & Melchior, 1983) or ATP (Hebert & Carruthers, 1986). While rather different methodologies and assumptions were adopted in these studies, it is difficult to rationalize these divergent findings. Jensen and Brahm (1987) formed ghosts by lysis of cells in 12 volumes of lysis medium, thus reducing intracellular ATP levels to about 170 μM. Hebert and Carruthers (1986) report that ATP increases the $K_{m(app)}$ for sugar exit half-maximally at 50 μM; thus, in the studies of Jensen and Brahm, the transport system would still by 77% saturated with endogenous ATP. This disagreement could result from methods of data analysis. Hebert and Carruthers (1986) employed the integrated rate equation for analysis of the $K_{m(app)}$ for exit using exit time course data obtained from radiolabeled and turbidimetry experiments while Jensen and Brahm (1987) employed a rapid quenching procedure to obtain initial rate exit data. Figure 1 of Jensen and Brahm (1987) shows the [D-glucose] dependence of zero-trans and equilibrium exchange exit from fresh and outdated red blood cells. The net exit data at high $[\text{D-glucose}]_i$ with outdated blood are indistinguishable from those obtained with fresh blood, confirming unchanged V_{max}. However, the demonstrations of an altered $K_{m(app)}$ for net and exchange exit in outdated blood (Jensen & Brahm, 1987) are completely dependent upon data obtained from measurements of the net exit at two $[\text{D-glucose}]_i$ and measurements of exchange exit at a single subsaturating $[\text{D-glucose}]_i$. This is sufficient to skew a linear transformation of these data to obtain an altered $K_{m(app)}$ but is insufficient to establish the statistical significance of these findings.

We examined the effects of Ca^{2+} on transport in intact cells and ghosts as previous studies from our laboratory have employed solutions containing EGTA or EDTA whereas other studies employed EGTA- and EDTA-free, phosphate-buffered media. We have described a small inhibitory action of phosphate-buffered medium versus EGTA-containing, Tris-buffered KCl medium on transport that could result from the presence of trace (micromolar) levels of Ca^{2+} or from the absence of EGTA. Ca^{2+} contamination of chemicals, water, and glassware/plasticware can be significant (Baker & Carruthers, 1983). In the absence of detailed knowledge of contaminating Ca^{2+} levels in previous studies, this hypothesis remains speculative. This current study confirms that Ca^{2+}_i and Ca^{2+}_o act to inhibit glucose transport in intact cells, ghosts, and ghosts containing ATP. The inhibitory actions of both extra- and intracellular Ca^{2+} are blunted in the presence of ATP_i. Our estimates of the $K_{i(app)}$ for transport inhibition by external and internal Ca^{2+} (2 μM) are consistent with the view that under physiological conditions [$[Ca]_o$ = 1 mM, $[Ca^{2+}]_i$ = 0.5 μM; see Simons 1982)] red cell sugar transport is tonically inhibited.

Our studies are consistent with those of Jacquez (1983) indicating that Ca^{2+} plus ionophore A23187 inhibits the V_{max} for D-glucose uptake by red cells at 4 °C. The same study (Jacquez, 1983) indicated that lysis and resealing of ionophore-treated cells were without effect on inhibited transport but that resealing ghosts (from ionophore-treated cells) in the presence of ATP reversed transport inhibition. In addition, incubation of Ca ionophore treated cells with medium containing metabolic substrates reversed ionophore inhibition of RBC glucose uptake. Jacquez (1983) interpreted these findings to be consistent with the view that ionophore plus Ca^{2+} treatment of red cells results in metabolic depletion which, owing to the suggested stimulation of transport by ATP, results in transport inhibition. An alternative explanation of these results is that raised intracellular $[Ca^{2+}]$ results in sugar transport inhibition which is reversed upon restoration of normal cellular metabolism (e.g., by feeding cells or by forming ghosts in the presence of ATP).

As ionophore plus Ca^{2+} also cause cellular ATP depletion, this alternative model predicts that removal of Ca^{2+} in depleted cells would not reverse transport inhibition by A23187 plus Ca^{2+}, an experimental finding confirmed by Jacquez (1983). Our studies also support this alternative hypothesis. Ca inhibition of transport is blunted by intracellular ATP and is reversible in intact cells and ghosts containing ATP but is not reversed in ghosts lacking ATP.

Sugar transport in avian erythrocytes is stimulated by metabolic depletion (Simons, 1983). Simons (1983) has demonstrated that stimulation of protein-mediated 3OMG uptake following 4 h of CN treatment to reduce total $[ATP]_i$ from 3 mM to 70 μM is reduced by the presence of extracellular Ca^{2+}. Interpretation of ionophore effects on transport is not simple, however, as A23187 in the absence of Ca can act to inhibit transport by a poorly understood mechanism (Simons, 1983; Bihler et al., 1980).

The mechanism of Ca inhibition of glucose transport is unknown at this time. Inhibition by Ca^{2+} is observed in substantially ATP-free ghosts, but it is possible that sufficient endogenous ATP is present [3 ± 1 μM (Hebert & Carruthers, 1986)] to allow a Ca^{2+}-dependent kinase inhibition of transport. This seems unlikely given that inhibition of sugar uptake by extracellular Ca in EGTA-containing, nominally ATP- and Ca-free ghosts is unaffected by the presence of 2 mM intracellular AMPPNP. One possibility is Ca activation of an as yet unidentified RBC phospholipase A_2 type activity to generate transport-inhibitory bilayer free fatty acids, and lysolipids. This possibility is consistent with the demonstrations of Ca-dependent, exogenous phospholipase A_2 inhibitions of sugar transport in red cells (Fujii et al., 1986), but we observe no detectable effect of intracellular or extracellular $[Ca^{2+}]$ on red cell total lysolipid levels. A general effect on bilayer lipid cannot be excluded. Red cell lysis and resealing in the presence of Ca^{2+} reduce bilayer lipid asymmetry (Schlegel et al., 1985). Primate red blood cell bilayer lipid asymmetry is reduced following incubation in glucose-free saline (Alam, 1985). This effect can be prevented by addition of EGTA or glucose to saline (Alam, 1985). Ca^{2+} plus A23187 treatment of human red cells has been demonstrated to decrease the protein band 4.1 content and to increase the band 2.3 content of red cell membranes (Allan & Thomas, 1981). In addition membrane polyphosphoinositide, phosphatidate, and 1,2-diacylglycerol contents are decreased, increased, and slightly increased, respectively, by Ca^{2+} plus A23187 treatment of normal RBCs whereas Ca^{2+} plus A23187 treatment of metabolically depleted red cells is without effect on the phosphatidate content of the membrane but increases the diacylglycerol content of the membrane (Allan & Thomas, 1981). Ca^{2+} elevation of 1,2-diacylglycerol (DAG) may, therefore, inhibit glucose transport, an effect reversed by phosphorylation of DAG to form phosphatidic acid. However, we observe no significant effects of $[Ca^{2+}]_o$ (100 μM) on the total phosphatidate or DAG

content of red cell membranes. Neither is red cell membrane band 4.1 protein content affected by the treatments (at physiological ionic strength) employed in this study (not shown).

Conclusions. Our studies support the hypothesis that ATP interacts in an allosteric manner with the sugar transport system of human erythrocytes to modify the $K_{m(app)}$ and V_{max} for sugar uptake (Carruthers, 1986). In addition, ATP can blunt and even reverse ATP-independent sugar transport inhibitions produced by intra- and extracellular Ca^{2+}. Evidence is presented suggesting that ATP reversal of Ca^{2+} inhibition of transport requires ATP hydrolysis. This point remains to be demonstrated directly. The mechanism of Ca^{2+} inhibition of transport is unknown. The effect persists in ghosts from Ca-treated cells but can be reversed by ATP_i, suggesting a Ca-induced, reversible, compositional or structural modification of the red cell membrane. These findings indicate that interpretation of the effects of pharmacologic manipulation of red cells, red cell lysis, and resealing or prolonged cold storage on sugar transport rates in erythrocytes may be complicated by effects of altered Ca^{2+} and ATP levels on the transport process.

Registry No. 5′-ATP, 56-65-5; AMPPNP, 25612-73-1; Ca, 7440-70-2.

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