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Biochemical Pharmacology 69 (2005) 1257–1262
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

cGMP (guanosine 3′,5′-cyclic monophosphate) transport across human erythrocyte membranes

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Received 17 December 2004; accepted 9 February 2005

Abstract

Human erythrocytes produce cGMP that can be eliminated by phosphodiesterases or active efflux transporters. The efflux can be studied under controlled conditions as ATP-dependent uptake into inside-out membrane vesicles. However, widely differing values for the transport rates have been reported. We have here examined factors that influence the uptake rates measured and thus may explain these discrepancies. Both the ionic composition of the buffer used during uptake and the mode of vesicle preparation were found to affect the observed transport rates. Furthermore it was apparent that different blood donors expressed on their erythrocytes different amounts of both MRP4 and MRP5, transporters that have been putatively linked to cGMP efflux across erythrocyte membranes. These differences in expression were reflected in differences in rates of cGMP uptake into inside-out erythrocyte membrane vesicles. Calculations based on the transport rates observed using vesicles suggest that efflux may be the principal means for eliminating cGMP from human erythrocytes.

Keywords: Erythrocyte; cGMP; ABC transporter; Multidrug-resistance associated protein; Membrane vesicles; MRP4

1. Introduction

It is well recognized that human red blood cells can produce cGMP (see e.g. [1]). Intracellular levels of this cyclic nucleotide may be important for regulation of red cell membrane viscosity [2], cell deformability [3] and under some circumstances may directly affect ion transport [1,4–6]. cGMP may also affect the breakdown of cAMP that in turn has been shown to affect red cell ion transport activities [7–10]. Intracellular cGMP can be broken down by phosphodiesterases [1,4,11] or it can be actively effluxed (see [12], for a review). The relative importance of these two disposal mechanisms in red cells has not been established and it is possible that active efflux plays a major role.

Studies with intact erythrocytes have demonstrated the existence of both an active efflux process and an uptake process that appears to be passive, driven by the concentration gradient of cGMP [13]. The kinetics of the active transport have been analysed using inside-out membrane vesicles prepared using several different methods [14–18]. In a previous study [18], we reported that the active uptake of cGMP consists of one or possibly two components: a low affinity component (dissociation constant 50–85 μM), and a much smaller high affinity component (dissociation constant 0.5–2.5 μM). Components with similar affinities have been reported by Sager et al. [12,14,19]. However in their studies the high-affinity component was relatively more prominent, the transport rates at all cGMP concentrations being much smaller. The discrepancy is of considerable importance because the lower rates reported by Sager et al. would, when scaled to the size of the erythrocyte, be too low to regulate the intracellular concentration of cGMP (see Section 4). The experimental methods used by the two laboratories differ in a number of respects. We report here the effects these differences have on the extent of uptake and show that both the mode of vesicle preparation and the composition of uptake buffer used influence the ATP-dependent transport of cGMP measured in human erythrocyte membrane vesicles.

We have also examined the level of expression of some transporters known to be capable of transferring cGMP. These are the multidrug-resistance associated proteins, MRP4 and MRP5, both of which have been detected on red blood cell membranes. Our results suggest that, in

Abbreviations: OSV, one-step inside-out membrane vesicles prepared by the spontaneous vesiculation method; SKNV, inside-out membrane vesicles prepared by the modified Steck and Kant; Nycodenz method

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doi:10.1016/j.bcp.2005.02.005

addition to methodological differences, variations in expression of these transporters in red cells derived from different individuals can produce variations in transfer rates of cGMP across red cell membranes.

2. Materials and methods

2.1. Chemicals

[8-^{3}H]cGMP (specific activity 13.9 Ci mmol^{-1}) was obtained from Amersham Biosciences. Adenosine triphosphate (ATP), ATP-γ-S, creatine phosphokinase, creatine kinase and cGMP were all obtained from Sigma Chemicals. M_5I-1 mAb was a kind gift of R.J. Scheper (Free University, Amsterdam, The Netherlands); anti-MRP4 mAb was a kind gift of G.D. Kruh (Fox Chase Cancer Centre, Philadelphia, USA). M_5I-1 and anti-MRP4 mAbs have been previously described [20,21].

2.2. Preparation of one-step vesicles, OSV

Fresh venous blood was drawn from normal healthy individuals into heparinized syringes and processed immediately. Membrane vesicles were prepared according to a procedure previously described [18,22,23]. Red blood cells were washed four times by centrifugation with 5 volumes of isotonic medium (70 mM NaCl; 80 mM KCl; 0.2 mM $MgCl_2$; 10 mM HEPES and 0.1 mM EGTA, pH 7.5) and removal of the supernatant and buffy coat. The packed red cells were then lysed in 90 volumes of ice-cold lysis solution (2 mM HEPES and 0.1 mM EGTA, pH 7.5) followed by 20 min of centrifugation at 40,000 × g at 4 °C. The supernatant was removed and the pelleted ghosts were resuspended in ice-cold lysis solution. The same step was repeated three times. Finally, the pelleted ghosts were resuspended in ice-cold lysis solution (half volume of the original blood sample) and incubated at 37 °C for 30 min to allow spontaneous vesiculation [23]. The ghosts were vortexed several times during this incubation. Approximately 30–37% of the vesicles are inside-out [18]. The resulting suspension was washed several times with the lysis solution to remove the supernatant including spectrin and actin. The pellet was then resuspended in 10 mM Tris–HCl solution, pH 7.4 and stored in aliquots at −80 °C until use. The protein concentrations of the vesicle samples were determined using the BCA (bicinchoninic acid) protein assay (Pierce).

2.3. Preparation of Steck and Kant, Nycodenz vesicles: SKNV

Inside-out membrane vesicles were prepared according to the procedure detailed previously [24] with the addition of a final Nycodenz purification step [14,16]. Briefly, venous blood (10 ml) was sampled in EDTA/heparin tubes and washed three times with five volumes of buffer A (110 mM NaCl, 3 mM KCl and 5 mM Tris–HCl; pH 8.0) by centrifugation (1000 × g for 15 min) with removal of the buffy coat at each wash. All subsequent procedures were carried out either on ice or at 4 °C. Cells (4 ml packed cells in 20 ml buffer A) were lysed with buffer B (3 mM KCl and 5 mM Tris–HCl; pH 8.0). The ghosts were sedimented (20,000 × g for 10 min) and resuspended (1 ml in 20 ml buffer B) three times resulting in milky white membranes and a clear final supernatant. Loosely packed ghosts were removed without the small hard button (rich in contaminating proteases). One milliliter of ghost pellet was resuspended in 39 ml of 0.5 mM Tris–HCl (pH 8.0), and left for 2 h at 0–4 °C. The membranes were sedimented (100,000 × g for 30 mins), then mixed with 1 ml of 0.5 mM Tris–HCl (pH 8.0) and vesiculated by passing them five times through a 20 mm-long 27-gauge needle. The mixture was diluted with 1 ml of 0.5 mM Tris–HCl (pH 8.0), layered onto a linear gradient (1.05–1.15 g ml^{-1}) of Nycodenz (Nycomed Pharma) and centrifuged at 100,000 × g, 4 °C for 16 h. The layer containing vesicles was washed, diluted with uptake buffer and frozen until use.

2.4. Vesicle uptake studies

ATP-dependent transport of radiolabelled substrates into membrane vesicles was measured by a rapid filtration technique [25,26]. For the [^{3}H]cGMP uptake studies, thawed membrane vesicles from human erythrocytes were added to a buffer system (55 μl final volume) containing 1 mM ATP, 10 mM $MgCl_2$, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase, 10 mM Tris–HCl (pH 7.4) and 3.3 μM [^{3}H]cGMP. After 15 min (or other times to measure the time course) at 37 °C incubation, aliquots of 20 μl were taken from the mixture (typically 15 min for [^{3}H]cGMP uptake studies), added into 1 ml of ice-cold stop solution (10 mM Tris–HCl, pH 7.4) and subsequently filtered through nitrocellulose filters (Whatman 0.2 μm pore size). All filters were pre-soaked overnight in 3% (w/v) bovine serum albumin to reduce non-specific binding of radiolabelled substrate. The filters were rinsed with at least 3 ml of ice-cold stop solution and the tracer retained on the filter determined by liquid scintillation counting. The background for each uptake study was measured using the non-hydrolysable ATP analogue, ATP-γ-S in the presence of the ATP regenerating system (10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase). This background value was subtracted from the values obtained in the presence of ATP to provide values of ATP-dependent uptake.

2.5. SDS-PAGE and Western blotting

Membrane vesicle or crude lysate proteins were separated through 7.5% (w/v) polyacrylamide and subsequently transferred onto Hybond ECL nitrocellulose

membranes (Amersham Bioscience). Each membrane was then incubated in blocking buffer [5% (w/v) milk powder in 0.1% TBS-Tween (25 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.1% Tween-20)] overnight at 4 °C prior to the addition of the primary Ig (M_5I-1, 1:40 dilution; anti-MRP4, 1:300 dilution). The positions of the MRP proteins on the membranes were visualized using the enhanced chemiluminescence horseradish peroxidase (HRP) detection system (Amersham Biosciences). The secondary antibodies used were the HRP-conjugated rabbit anti-(rat IgG) Ig (1:2000 dilution for M_5I-1) or HRP-conjugated rabbit anti-(mouse IgG) Ig (1:2000 dilution for anti-MRP4).

3. Results

3.1. ATP-dependent uptake of cGMP into one-step human erythrocyte membrane vesicles, OSV

In agreement with previous work [18] the 15 min uptake of 3.3 μM [^{3}H]cGMP at 37 °C into OSV (inside-out vesicles prepared from human erythrocytes using the one-step procedure) in the presence of ATP was more than 20 times greater than when the ATP was replaced by the non-hydrolysable ATP analogue, ATP-γ-S. The ATP-dependent uptake rate, calculated from the difference in uptakes, remained essentially constant over the course of 30 min. The variation of uptake rate with cGMP concentration is shown in Fig. 1. The results obtained using OSV differ markedly from that found previously using SKNV (inside-out vesicles prepared from human erythrocytes using the Steck and Kant, Nycodenz procedure) [14,16,19] (see Fig. 1) in that the measured uptake rates per milligram of membrane protein for OSV are ∼40-fold higher than for SKNV even at low concentrations. The data for OSV have been fitted using the equations for a single low affinity component of uptake. The improvement in fit obtained by allowing for a second high affinity component did not reach statistical significance. The smaller, high affinity transport reported by Sager et al. [12,14,19] would not have been resolved from the low affinity component by these fits and thus may be present in these vesicles.

3.2. Effect of incubation buffer on ATP-dependent uptake of cGMP into OSV

Previous experiments with SKNV used an isotonic NaCl/phosphate buffer which differs from the lower osmolality Mg–Tris Cl buffer (10 mM $MgCl_2$, 10 mM Tris–HCl, 1 mM ATP, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase; pH 7.4) employed in experiments with OSV. A direct comparison of uptake rates obtained with the NaCl/phosphate buffer and the Mg–Tris buffer is shown in Fig. 2 for OSV (see Fig. 2). In the presence of PBS, the [^{3}H]cGMP uptake rate was reduced by approximately 50% at all cGMP concentrations used.

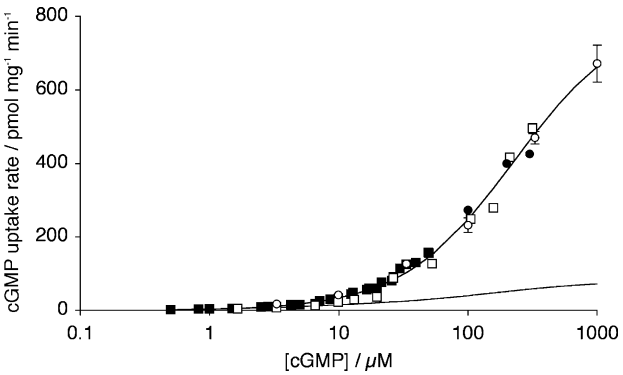

Fig. 1. ATP-dependent uptake of cGMP into inside-out membrane vesicles prepared from human erythrocytes. The graph shows the relation between uptake rate and cGMP concentration in the presence of 1 mM ATP (after subtraction of the much smaller uptake observed when ATP is replaced by ATP-γ-S). Data are reported for four separate experimental series using vesicles prepared by the one step vesiculation method (OSV). All values within a series are multiplied by a scale factor, SF, for that series to allow for differences between donors and yield of the vesicle preparation. Filled squares are the original series (replotted from [18]) for concentrations up to 50 μM ($SF_1 = 1$). Filled circles are from a later series (replotted from [18]) to provide values for concentrations of 100 μM and higher ($SF_2 = 1.49$). Open squares ($SF_3 = 1.07$) and open circles ($SF_4 = 2.13$) are new data. The fitted curve is drawn assuming a single component with $K_d = 230$ μM and $U_{max}/K_d \sim 3.5$ pmol mg^{-1} min^{-1} μM^{-1}. Each point represents the mean of at least three determinations in at least two independent experiments (including independent vesicle preparation). Error bars represent S.E.M.; where not visible, they are within the size of the symbol. For comparison, the uptake rates predicted from the parameters reported by Sager et al. [19] using vesicles prepared by the Steck and Kant method (SKNV) are shown scaled upwards 40 fold ($SF_S = 40$) as the lower line.

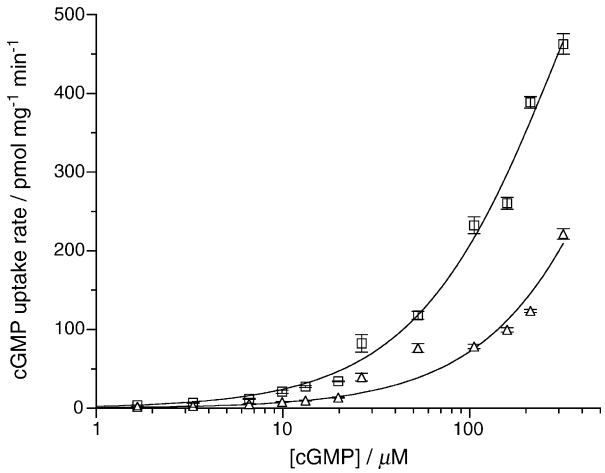

Fig. 2. Effect of buffer ionic composition on the rate of ATP-dependent cGMP transport into one-step inside-out erythrocyte membrane vesicles (OSV). The cGMP uptake rate was measured using either (triangles) an isotonic Na/phosphate buffer solution (140 mM NaCl; 3 mM KCl; 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 10 mM $MgCl_2$ and 2 mM ATP; pH 7.4) or (squares) a lower osmolality Mg/Tris uptake buffer (10 mM $MgCl_2$, 10 mM Tris–HCl, 1 mM ATP, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase; pH7.4). The uptake rate was 2–3-fold lower using the Na/phosphate solution. All data points represent at least three independent experiments from a single vesicle preparation.

3.3. Comparison of cGMP uptake into OSV and SKNV

To determine whether the one-step method and the Steck and Kant, Nycodenz method produced vesicles that display different uptake rates, a direct comparison of rates measured using the same assay conditions, i.e. Mg–Tris Cl uptake buffer, was undertaken using vesicles prepared by the two separate methods from fresh blood taken from a single individual. ATP-dependent uptake of 3.3 μM cGMP into SKNV was found to be markedly less than into OSV (see Fig. 3). At 330 μM cGMP the ATP-dependent uptakes at 15 min were 85 ± 13 pmol mg^{-1} for SKNV and 3300 ± 100 pmol mg^{-1} for OSV.

3.4. MRP4 and MRP5 expression in erythrocytes from different donors: implications for cGMP uptake into OSV

Immunodetection was used to determine the inter-individual variation in expression in human erythrocytes of the MRPs putatively linked to cyclic nucleotide transport. Erythrocyte membranes from 10 volunteers were prepared and immunoblot analysis was performed using the monoclonal antibodies anti-MRP4 and M_5I-1 for the detection of MRP4 and MRP5 proteins respectively (Fig. 4A). M_5I-1 specifically detected N-glycosylated MRP5 as an intact band at ∼190 kDa in erythrocytes. The anti-MRP4 antibody detected a moderate intact band at ∼180 kDa as N-glycosylated MRP4 in human erythrocytes as reported previously [18]. Some variation in expression between the individual blood donors was seen. To ascertain whether the different levels of expression were reflected in differences in cGMP transport, blood from the two individuals with the most contrasting levels of expression (A and B in Fig. 4A) were chosen for further functionality studies. As is evident in Fig. 4A, expression of both MRP proteins was noticeably lower in the erythrocyte membranes taken from donor B (lane 4) than in those taken from donor A (lane 1).

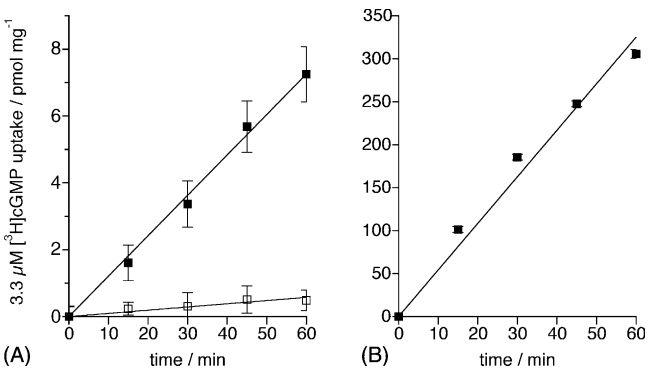

Fig. 3. Comparison of ATP-dependent uptake of cGMP into SKNV and OSV. Time dependent uptake of 3.3 μM cGMP into (A) SKNV and (B) OSV in the presence of 1 mM ATP (filled squares) or the non-hydrolysable analogue ATP-γ-S (open squares—not shown with OSV). Both types of vesicles were prepared from the same sample of blood and uptake was measured using the same Mg/Tris uptake buffer.

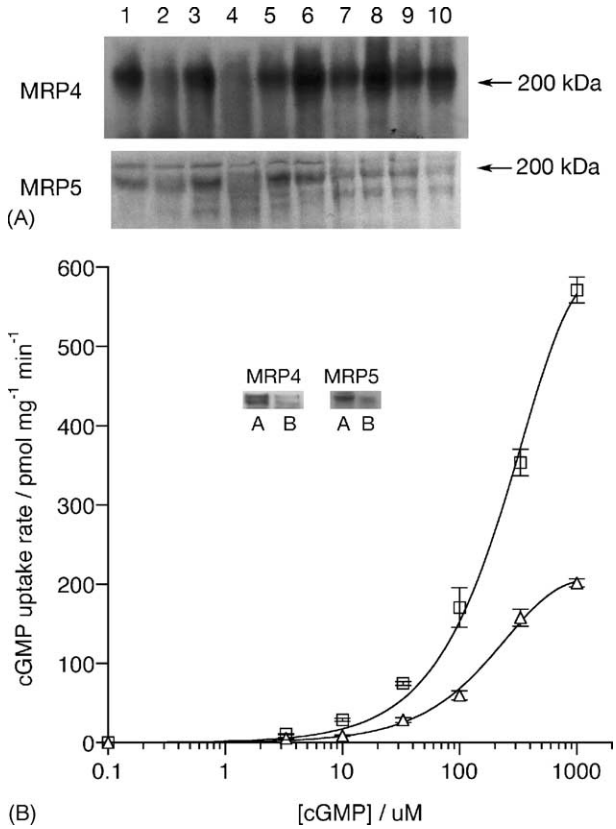

Fig. 4. Inter-individual variations in MRP4 and MRP5 expression in human erythrocytes: implications for cGMP uptake into inside-out erythrocyte membrane vesicles. (A) Immunodetection of MRP4 and MRP5 in human erythrocyte membranes from 10 donors. Freshly drawn human blood from 10 individuals was taken and the expression of each MRP was detected with specific antibodies as detailed in Section 2. Forty micrograms of equivalent of protein sample was loaded in each lane (equal loading was confirmed by Ponceau red staining). Expression levels are seen to vary several fold. Donor A (lane 1) was chosen as the "higher than average" individual whilst donor B (lane 4) was chosen as "lower than average". Representative blots are shown ($n = 3$). (B) Direct comparison of MRP-mediated cGMP uptake into inside-out membrane vesicles (OSV) prepared from human erythrocytes of donors A and B. Membrane vesicles containing 50 μg protein were used for each of the uptake studies. The cGMP uptake rate achieved with vesicles from donor A (squares) was ∼2-fold higher than donor B (triangles). Data for each donor were determined in at least three independent experiments at least one from each of the two separate vesicle preparations.

Blood from these donors was used to prepare inside-out membrane vesicles via the one-step spontaneous vesiculation method and then both high affinity (3.3 μM) and low affinity (330 μM) ATP-dependent uptake of [^{3}H]cGMP were measured. The apparent cGMP uptake rates achieved in vesicles derived from donor A blood were approximately 2-fold higher than those seen in vesicles derived from donor B blood (Fig. 4B).

4. Discussion

Uptake studies using membrane vesicles have important advantages for investigations of active efflux transporters in red blood cells. The passive components of transport and

binding to unsealed vesicles can be excluded from the analysis by subtraction of uptake observed in the absence of ATP or when it is replaced by a non-hydrolyzable analogue. Only active uptake into inside-out vesicles is measured. Active transporters in right-side out vesicles do not contribute since their ATP binding domains are inside the vesicles and thus not accessible to externally applied ATP. It has been shown previously that inside-out membrane vesicles prepared from human erythrocytes by either the Steck and Kant, Nycodenz method [14,19,24] (SKNV) or by a one-step spontaneous vesiculation method [18,22,23] (OSV) are able to take up cGMP in a temperature and ATP dependent manner [14,16,18]. However, the maximum uptake rates reported in the previous study using OSV [18] were more than 100-fold greater than those reported in the studies using SKNV (see Fig. 1).

While a number of factors may contribute [18], we find here that the different choice of buffer solutions for measuring the uptakes, the different methods of vesicle preparation and use of blood from different donors can together account for most of the discrepancies in the rates. The different assay buffers account for a 2-fold difference (see Fig. 2). Much larger differences are observed between uptakes measured with membrane vesicles prepared in the different ways i.e. between SKNV and OSV, ATP-dependent uptake rates at 3.3 μM were found to be more than 40-fold higher into OSV (see Fig. 3). In our hands most of the loss in transport activity in the SKNV appeared to happen at the last stage on passage through the Nycodenz column. The Steck and Kant procedure has been used successfully with red blood cells for measurement of both high and low affinity components for DNP-SG transport [27,28]. By contrast the Gardos channel (a Ca^{2+} activated K^+ channel) could not be demonstrated in Steck and Kant vesicles but was active in OSV [22].

The method of vesicle preparation and the choice of uptake buffer together can account for most of the differences in uptake rates between previous reports. An additional factor that might contribute is variation in expression of transporters in the erythrocytes i.e. differences between individual blood donors. This would account for the ~2-fold differences in uptake rates we have observed in different series of experiments (Fig. 1) using the same methods of preparation and assay. To investigate this further we looked at blood samples drawn from a panel of 10 donors and red blood cells from the two donors displaying the largest differences in expression were selected for a head-to-head study (see Fig. 4). The difference in expression of both MRP4 and MRP5 was mirrored by a difference in the uptake rates into OSV prepared from the cells consistent with an important role for either MRP4 or MRP5 in cGMP transport in erythrocytes.

There is now extensive, though still not conclusive, evidence that MRP4 is the principal mediator of the low-affinity, ATP-dependent export of cGMP from red blood cells. MRP8 can also transport cGMP [29]. However two independent unpublished studies using three different antibodies have failed to find MRP8 in red blood cell membranes (N. Ono, P. Borst, personal communication, pMal-81-3 and M8II-16; C.-P. Wu, S.V. Ambudkar, personal communication, affinity purified rabbit polyclonal anti-human MRP8). Red blood cells also express MRP5 [18,30], which transports cGMP, and there is evidence [31] that immunoprecipitation of MRP5 decreases the transport activity of reconstituted protein extracts from red blood cell membranes. The presence of a second transporter with somewhat higher affinity would provide a ready explanation for the variations observed with different donors in the apparent dissociation constant of cGMP uptake (see Fig. 1). This idea is reinforced by the finding that mefloquine, PGA1 and PGE1 inhibit most but not all of the cGMP uptake into membrane vesicles formed by the one-step spontaneous procedure (Wu, Hladky and Barrand, unpublished observations) as if there were present two similar components of transport, but only one inhibitable by these agents.

For efflux to play a major role in the regulation of cGMP levels in red cells, it should satisfy two conditions: it must be fast enough that the concentrations will approach a new steady-state within a reasonable amount of time after a change in the rate of synthesis; and it should be fast enough at the cGMP concentrations found in the cells to balance the basal rate of secretion. The transport measured here and in our preceding work [18] is of low affinity with apparent dissociation constant (>100 μM) far greater than typical intracellular cGMP concentrations (e.g. 3.7 nM [1]). However high capacity, low affinity transport as a means of elimination has the advantage that it does not saturate and thus increases in the rate of cGMP production would produce only proportional increases in the steady-state intracellular concentration rather than the much larger, uncontrolled changes associated with saturable elimination. The efflux rate at low concentrations predicted from the vesicle results can be significant (U_{max}/K_d is ~3.5 pmol mg^{-1} min^{-1} μM^{-1} in the inside-out vesicles which should be multiplied by ~3 to account for the proportion of vesicles that are inside-out [18]). Thus the rate of efflux from a single cell (10^{-9} mg of membrane protein, 80 μm^3 volume) will exceed ~10^{-20} mol min^{-1} μM^{-1} × intracellular concentration of cGMP. Even if there were no other routes of removal of cGMP, the half-time for changes in concentration following a change in rate of synthesis would thus be less than about 5 min. Thus the first condition is satisfied.

A direct test of the second condition is not yet possible as there are no direct determinations of the basal rate of production that can be compared with the rate of efflux. Indirect evidence for the importance of efflux comes from studies investigating the inhibition of the phosphodiesterases. Petrov and Lijnen [1,4] found that inhibition of phosphodiesterases could at most double the basal levels, the expected result if both efflux and hydrolysis were involved roughly equally in the basal elimination of cGMP. Evidence for the relative lack of importance of hydrolysis

is the lack of significant formation of hydrolysis products in experiments involving prolonged loading of cells with [^{3}H]cGMP [13].

Both NO acting on soluble guanylate cyclase and atrial natriuretic peptide acting via membrane bound guanylate cyclase can increase cGMP levels in human red blood cells [1]. As noted in the introduction these, increases are likely to be of physiological significance. The concentrations of cGMP achieved in red blood cells obviously result from a balance between synthesis and removal and so the rate of removal is equally as important as the rate of production. We report here that in red blood cells a major part of this removal can be by efflux from the cells and that the ability to efflux cGMP in erythrocytes varies between donors.

Acknowledgement

C.P. Wu is supported partially by funds from a Cambridge Commonwealth Trust Bursary.

References

[1] Petrov V, Lijnen P. Regulation of human erythrocyte Na^+/H^+ exchange by soluble and particulate guanylate cyclase. Am J Physiol Cell Physiol 1996;271:C1556–64.
[2] Tsuda K, Kinoshita Y, Kimura K, Nishio I, Masuyama Y. Electron paramagnetic resonance investigation on modulatory effect of 17 beta-estradiol on membrane fluidity of erythrocytes in postmenopausal women. Arterioscler Thromb Vasc Biol 2001;21:1306–12.
[3] Bor-Kucukatay M, Wenby RB, Meiselman HJ, Baskurt OK. Effects of nitric oxide on red blood cell deformability. Am J Physiol Heart Circ Physiol 2003;284:H1577–84.
[4] Petrov V, Fagard R, Lijnen P. Human erythrocytes contain Ca^{2+}, calmodulin-dependent cyclic nucleotide phosphodiesterase which is involved in the hydrolysis of cGMP. Meth Find Exp Clin Pharmacol 1998;20:387–93.
[5] Anthony TL, Brooks HL, Boassa D, Leonov S, Yanochko GM, Regan JW, et al. Cloned human aquaporin-1 is a cyclic GMP-gated ion channel. Mol Pharmacol 2000;57:576–88.
[6] Boassa D, Yool AJ. Single amino acids in the carboxyl terminal domain of aquaporin contribute to cGMP-dependent ion channel activation. BMC Physiol 2003;3:12.
[7] Kim HD. Ion-transport and adenylyl-cyclase system in red-blood-cells. Curr Top Membr 1991;39:181–225.
[8] Pellegrino R, Pellegrini M. Modulation of Ca^{2+}-activated K^+ channels of human erythrocytes by endogenous cAMP-dependent protein kinase. Pflugers Arch 1998;436:749–56.
[9] Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. Participation of cAMP in a signal-transduction pathway relating erythrocyte deformation to ATP release. Am J Physiol Cell Physiol 2001;281:C1158–64.
[10] Soldati L, Adamo D, Spaventa R, Bianchi G, Vezzoli G. Chloride fluxes activated by parathyroid hormone in human erythrocytes. Biochem Biophys Res Commun 2000;269:470–3.
[11] Sheppard H, Burghardt CR. The stimulation of adenyl cyclase of rat erythrocyte ghosts. Mol Pharmacol 1970;6:425–9.
[12] Sager G. Cyclic GMP transporters. Neurochem Int 2004;45:865–73.
[13] Flo K, Hansen M, Orbo A, Kjorstad KE, Maltau JM, Sager G. Effect of probenecid, verapamil and progesterone on the concentration-dependent and temperature-sensitive human erythrocyte uptake and export of guanosine 3′,5′-cyclic monophosphate (cGMP). Scand J Clin Lab Invest 1995;55:715–21.
[14] Schultz C, Vaskinn S, Kildalsen H, Sager G. Cyclic AMP stimulates the cyclic GMP egression pump in human erythrocytes: Effects of probenecid, verapamil, progesterone, theophylline, IBMX, forskolin, and cyclic AMP on cyclic GMP uptake and association to inside-out vesicles. Biochemistry 1998;37:1161–6.
[15] Vaskinn S, Sundkvist E, Jaeger R, Sager G. The effect of Mg2+, nucleotides and ATPase inhibitors on the uptake of H-3-cGMP to inside-out vesicles from human erythrocytes. Mol Membr Biol 1999;16:181–8.
[16] Sundkvist E, Jaeger R, Sager G. Leukotriene C4 (LTC4) does not share a cellular efflux mechanism with cGMP: Characterisation of cGMP transport by uptake to inside-out vesicles from human erythrocytes. Biochim Biophys Acta Biomembr 2000;1463:121–30.
[17] Sundkvist E, Jaeger R, Sager G. Pharmacological characterization of the ATP-dependent low K-m guanosine 3′,5′-cyclic monophosphate (cGMP) transporter in human erythrocytes. Biochem Pharmacol 2002;63:945–9.
[18] Klokouzas A, Wu CP, Van Veen HW, Barrand MA, Hladky SB. cGMP and glutathione-conjugate transport in human erythrocytes—the roles of the multidrug resistance-associated proteins, MRP1, MRP4 and MRP5. Eur J Biochem 2003;270:3696–708.
[19] Sager G, Orbo A, Pettersen RH, Kjorstad KE. Export of guanosine 3′,5′-cyclic monophosphate (cGMP) from human erythrocytes characterized by inside-out membrane vesicles. Scand J Clin Lab Invest 1996;56:289–93.
[20] Scheffer GL, Kool M, Heijn M, de Haas M, Pijnenborg A, Wijnholds J, et al. Specific detection of multidrug resistance proteins MRP1, MRP2, MRP3, MRP5, and MDR3 P-glycoprotein with a panel of monoclonal antibodies. Cancer Res 2000;60:5269–77.
[21] Lee K, Klein-Szanto AJP, Kruh GD. Analysis of the MRP4 drug resistance profile in transfected NIH3T3 cells. J Nat Cancer Inst 2000;92:1934–40.
[22] Lew VL, Muallem S, Seymour CA. Properties of the Ca^{2+}-activated K^+ channel in one-step inside-out vesicles from human red cell membranes. Nature 1982;296:742–4.
[23] Lew VL, Hockaday A, Areeman CJ, Bookchin RM. Mechanism of spontaneous inside-out vesiculation of red cell membranes. J Cell Biol 1988;106:1893–901.
[24] Steck TL, Kant JA. Preparation of impermeable ghosts and inside-out vesicles from human erythrocyte membranes. Meth Enzymol 1974; 31:172–80.
[25] Ishikawa T. ATP/Mg^{2+}-dependent cardiac transport system for glutathione *S*-conjugates. A study using rat heart sarcolemma vesicles. J Biol Chem 1989;264:17343–8.
[26] Ishikawa T. The ATP-dependent glutathione *S*-conjugate export pump. Trends Biochem Sci 1992;17:463–9.
[27] Akerboom TPM, Bartosz G, Sies H. Low- and high-K(m) transport of dinitrophenyl glutathione in inside out vesicles from human erythrocytes. Biochim Biophys Acta Biomembr 1992;1103:115–9.
[28] Pulaski L, Jedlitschky G, Leier I, Buchholz U, Keppler D. Identification of the multidrug-resistance protein (MRP) as the glutathione-*S*-conjugate export pump of erythrocytes. Eur J Biochem 1996;241:644–8.
[29] Guo YP, Kotova E, Chen ZS, Lee K, Hopper-Borge E, Belinsky MG, et al. MRP8, ATP-binding cassette C11 (ABCC11), is a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2′,3′-dideoxycytidine and 9′-(2′-phosphonylmethoxyethyl) adenine. J Biol Chem 2003;278:29509–14.
[30] Jedlitschky G, Burchell B, Keppler D. The multidrug resistance protein 5 functions as an ATP-dependent export pump for cyclic nucleotides. J Biol Chem 2000;275:30069–74.
[31] Boadu E, Sager G. Reconstitution of ATP-dependent cGMP transport into proteoliposomes by membrane proteins from human erythrocytes. Scand J Clin Lab Invest 2004;64:41–8.