*Biochimica et Biophysica Acta*, 1061 (1991) 305–308

*ADONIS* 000527369100086K



BBAMEM 70669

**BBA Report**

# Characteristics of $^{86}Rb^+$ transport in human erythrocytes infected with *Plasmodium falciparum*

Kiaran Kirk [1], Kevin J. Ashworth [1], Barry C. Elford [2], Robert A. Pinches [2] and J. Clive Ellory [1]

[1] *University Laboratory of Physiology, Oxford, (U.K.) and* [2] *Institute of Molecular Medicine, John Radcliffe Hospital, Oxford (U.K.)*

(Received 25 August 1990)

Key words: Rubidium-86 transport; Infected erythrocyte; (*P. falciparum*); (Human erythrocyte)

**Human red cells infected in vitro with *Plasmodium falciparum* showed a significant increase in the rate of both ouabain-sensitive and ouabain-insensitive $^{86}Rb^+$ influx. The increase in ouabain-insensitive $^{86}Rb^+$ influx was due, in part, to increased transport via a bumetanide-sensitive system and, in part to transport via a pathway that was absent (or at least inactive) in uninfected cells. The parasite-induced pathway was inhibited by piperine and had a dose response very similar to that of the Gardos channel of uninfected cells but was less sensitive than the Gardos channel to inhibition by quinine.**

More than 40 years ago, Overman [1] found that erythrocytes from monkeys infected with *Plasmodium knowlesi* showed an increased $Na^+$ and decreased $K^+$ content. Dunn [2] obtained similar results in blood obtained from animals infected with other species of malaria. In both of these studies $Na^+$ and $K^+$ content of the infected cells was measured using flame photometry and there was no attempt to differentiate between the ionic composition of the erythrocyte cytosol and that of the intracellular parasite itself. Recently however, Lee et al. [3] used X-ray microanalysis to examine the distribution of $Na^+$ and $K^+$ in human erythrocytes infected with *Plasmodium falciparum*. From this study it emerged that in cells infected with late-stage parasites, the parasites themselves maintain a high $K^+ : Na^+$ ratio (of nearly 8 : 1) within the parasitophorous vacuole but that in the erythrocyte cytoplasm the ratio is completely reversed, going from a value of more than 9 : 1 in uninfected cells to 1 : 12 in infected cells. Such a reversal indicates that the infection produces marked changes in the permeability of the erythrocyte membrane to $Na^+$ and $K^+$. In his original study Dunn [2] found a significant increase in the ouabain-insensitive 'leak' of $^{22}Na^+$ across the cell membrane. Similarly, Boockchin et al. [4] found an increase in the ouabain-insensitive transport of $^{42}K^+$ into infected cells. In the work reported here we have used $^{86}Rb^+$ as a $K^+$ congener, in conjunction with various transport inhibitors, to investigate the extent to which the endogenous $K^+$ transport pathways contribute to the altered $K^+$ properties of the parasitised erythrocyte.

Erythrocytes infected with the ITO4 line of *Plasmodium falciparum* were cultured as described previously [5] in RPMI 1640 ($[K^+] = 5.36$ mM) with 10% human serum under reduced oxygen tension (1% $O_2$, 3% $CO_2$, 96% $N_2$). The culture medium was supplemented with D-glucose (10 mM), glutamine (2 mM) and gentamycin sulphate (25 mg · $L^{-1}$). Synchronous cultures containing up to 80% parasitised erythrocytes with 90–95% at the mature pigmented trophozoite stage (30–35 h post-invasion) were obtained by a combination of gelatin flotation [6] and sorbitol lysis [7]. Suspensions of uninfected erythrocytes from the same donor (type O) were incubated in parallel with the infected red cell cultures. Cell concentrations were determined using a Coulter counter (Model Zm). Parasitaemia was estimated from Giemsa-stained smears.

In preparation for $^{86}Rb^+$ influx measurements, erythrocytes (both infected and uninfected) were washed four times then resuspended in RPMI (at 37°C) and combined with the appropriate reagent solutions in an Eppendorf tube to give a suspension having a final volume of 480 μl and a cell concentration of around $3 \cdot 10^8$ RBC · $ml^{-1}$. Samples were transferred to a 37°C water bath at least 10 min prior to beginning the influx incubation to ensure temperature-equilibration. $^{86}Rb^+$ influx was commenced by the addition to the suspension of a solution of $^{86}RbCl$ in RPMI (20 μl) to give a

Correspondence: K. Kirk, University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, U.K.

final activity of approx. 8 $\mu Ci \cdot ml^{-1}$. After an appropriate incubation period, $^{86}Rb^+$ influx was terminated by transferring three 140 $\mu$l aliquots of the suspension to separate Eppendorf tubes, each containing 800 $\mu$l of an ice-cold isotonic Mops-buffered $MgCl_2$ solution (106 mM $MgCl_2$, 15 mM Mops, pH 7.4) layered over 250 $\mu$l of dibutylphthalate. The tubes were centrifuged (10000 × g, 1 min) then the supernatant solution and dibutylphthalate were aspirated. Traces of solution on the inner wall of the tube were removed with cotton swabs. The cell pellet was lysed with 0.5% (v/v) Triton X-100 (0.5 ml) then deproteinised by the addition of 5% (w/v) trichloroacetic acid (0.5 ml) followed by centrifugation (10000 × g, 10 min). The radioactivity in the supernatant solution was counted using Cerenkov radiation.

In both malaria-infected and uninfected cells (in the absence of ionomycin), $^{86}Rb^+$ uptake was linear with time for up to 20 min, whether in the presence or absence of ouabain. In the experiments reported here (except those involving ionomycin) initial $^{86}Rb^+$ influx rates were estimated from the radioactivity accumulated during a 15–20 min incubation period. In uninfected cells to which ionomycin had been added the $^{86}Rb^+$ influx was much faster and initial $^{86}Rb^+$ influx rates were estimated from the radioactivity accumulated during a 4 min incubation period. The amount of radioactivity trapped in the extracellular space of the cell pellet was estimated in each experiment by adding the $^{86}RbCl$ solution (20 $\mu$l) to an icecold sample (480 $\mu$l) containing maximally effective concentrations of the appropriate transport inhibitors, then immediately separating the cells from the extracellular solution as has been described.

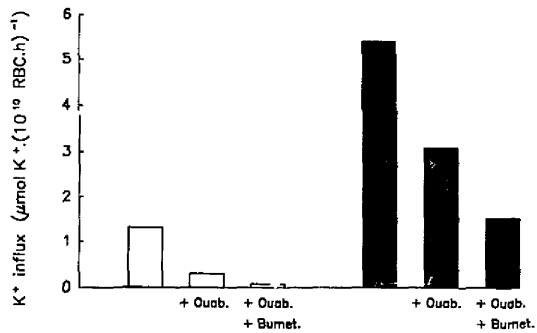


Fig. 1. The effect of ouabain (0.1 mM) and bumetanide (0.1 mM) on the rates of $K^+$ influx (calculated from the $^{86}Rb^+$ uptake) in uninfected erythrocytes (open bars) and in malaria-infected erythrocytes (84% parasitaemia, solid bars) taken from the same donor. The results are from a single experiment and are representative of those obtained in at least three such experiments on cells from different donors.

Fig. 1 shows the effects of ouabain (at a concentration sufficient to fully inhibit the $Na^+/K^+$ pump) and bumetanide (at a concentration sufficient to fully inhibit the NaKCl cotransport system) on the rate of $K^+$ transport in infected and uninfected erythrocytes. Table I shows the different components of $K^+$ influx in infected and uninfected cells from a number of different donors. Infection by the malaria parasite caused a significant increase in the ouabain-sensitive component ($P = 0.030$, paired $t$-test), the bumetanide-sensitive component ($P = 0.046$) and the residual, ouabain- and bumetanide-resistant component ($P = 0.025$) of $K^+$ influx.

Table I

*Ouabain-sensitive, bumetanide-sensitive and residual (ouabain- and bumetanide-resistant) $K^+$ influx in malaria-infected and uninfected erythrocytes from different donors*

Ouabain-sensitive $K^+$ influx was calculated from the decrease in $^{86}Rb^+$ uptake that accompanied the addition of ouabain (0.1 mM) to the cell suspensions. Bumetanide-sensitive $K^+$ influx was calculated from the decrease in $^{86}Rb^+$ uptake that accompanied the addition of bumetanide (0.1 mM) to cells pretreated with ouabain. The residual $K^+$ flux was calculated from the $^{86}Rb^+$ uptake measured in cells treated with both ouabain and bumetanide. Donors denoted A–D.

| Influx component | Donor | Parasitaemia (%) | $K^+$ influx ($\mu$mol $K^+/10^{10}$ RBC per h) | | Proportional increase |
|---|---|---|---|---|---|
| | | | malaria-infected cells | uninfected cells | |
| Ouabain-sensitive | A | 35 | 2.50 | 1.63 | 1.5 |
| | B | 56 | 2.36 | 1.69 | 1.4 |
| | C | 84 | 2.30 | 1.02 | 2.3 |
| | D | 62 | 1.89 | 1.57 | 1.2 |
| Bumetanide-sensitive | B | 56 | 1.55 | 0.48 | 3.2 |
| | C | 84 | 1.57 | 0.22 | 7.1 |
| | D | 62 | 0.70 | 0.11 | 6.4 |
| Residual | B | 56 | 2.04 | 0.09 | 21.5 |
| | C | 84 | 1.51 | 0.07 | 20.1 |
| | D | 62 | 1.24 | 0.12 | 10.3 |

The increase in the ouabain-sensitive component may presumably be attributed to an increase in the activity of the $Na^+/K^+$ pump, probably due to the raised intracellular $Na^+$ concentration (and decreased intracellular $K^+$ concentration) that has been shown to arise in erythrocytes infected by late-stage parasites [3]. $Na^+/K^+$ pump activity is a saturable function of $[Na^+]_i$ (the intracellular $Na^+$ concentration); it is half-maximal at around 12 mmol/L RBC $H_2O$ [8] which is close to the intracellular $Na^+$ concentration in uninfected cells. An increase in $[Na^+]_i$ will therefore cause the pump activity to increase by up to two-fold.

The significant increase in the bumetanide-sensitive component of $^{86}Rb^+$ uptake suggests that the activity of the NaKCl cotransport system was greater in malaria-infected cells. This increase may be attributed, in part, to the altered $[Na^+]_i$ and $[K^+]_i$; however, it was substantially larger than that which arises from a decrease in the $K^+ : Na^+$ ratio in normal human erythrocytes [9] and it therefore seems likely that other factors (such as the reduced intracellular pH or the substantially altered membrane composition of the infected cell) were involved.

The residual (ouabain- and bumetanide-resistant) $Rb^+$ influx in infected cells was inhibited by the alkaloids piperine and quinine. Neither compound caused any decrease in the much smaller residual $^{86}Rb^+$ uptake in uninfected cells which suggests that the pathway responsible for the enhanced $^{86}Rb^+$ uptake in the infected cells was absent from (or at least inactive in) uninfected erythrocytes. Quinine is an effective inhibitor of the Gardos channel, a high-capacity, $Ca^{2+}$-activated $K^+$ transport pathway that, in normal erythrocytes, is maintained in an inactive state by the very low intracellular $Ca^{2+}$ concentration [10]. The $Ca^{2+}$ content of malaria-infected erythrocytes is some 10–20 times higher than normal [4,11]. Although the major portion (80–90%) of the intracellular $Ca^{2+}$ in infected cells is thought to be localised within the parasite [11] it is possible that the concentration of $Ca^{2+}$ in the erythrocyte cytoplasm was raised sufficiently for there to have been significant activation of the Gardos channel in the parasitised cells.

Fig. 2 shows the dose–response curves for the effect of piperine and quinine on $^{86}Rb^+$ influx via the parasite-induced (ouabain- and bumetanide-resistant) pathway in infected cells and on $^{86}Rb^+$ influx via the Gardos channel which was activated (in uninfected cells) by the addition of the $Ca^{2+}$ ionophore ionomycin. There was a close similarity between the dose–response of the two pathways to piperine which produced a 50% inhibition of both systems at a concentration ($I_{50}$) of around 50 $\mu$M (Fig. 2a). In contrast, the dose–responses of the two pathways to quinine (Fig. 2b) were quite dissimilar. Quinine inhibited the Gardos channel with an $I_{50}$ of around 50 $\mu$M and blocked the channel completely at a concentration of 300 $\mu$M; the $I_{50}$ for the inhibition of the parasite-induced pathway by quinine was around 300 $\mu$M.

The observed difference in the sensitivity of the two pathways to inhibition by quinine becomes even more significant when it is considered in light of the finding by Reichstein and Rothstein [12] that, in normal human erythrocytes, an increase in $[Na^+]_i$ (with a corresponding decrease in $[K^+]_i$) causes a marked increase in the sensitivity of the Gardos channel to inhibition by quinine. On the basis of this result it might be expected that if the Gardos channel were activated in the infected

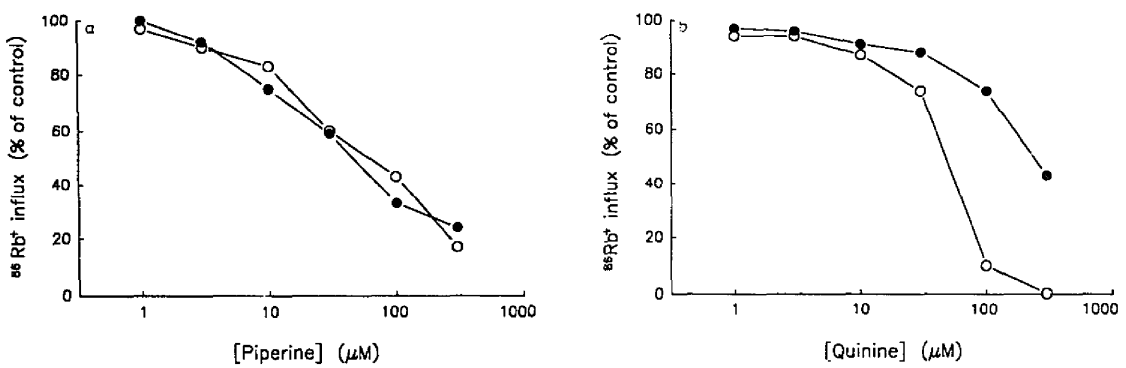


Fig. 2. Dose–response curves for the effects of (a) piperine and (b) quinine on $^{86}Rb^+$ influx via the parasite-induced (ouabain- and bumetanide-resistant) pathway in infected cells (closed symbols) and on $^{86}Rb^+$ influx via the $Ca^{2+}$-activated Gardos channel of uninfected cells (open symbols). Piperine was added to cells as a stock solution in DMSO and quinine as a solution of quinine sulphate in RPMI. All samples contained ouabain (0.1 mM) and bumetanide (0.1 mM). The Gardos channel was activated by the addition of ionomycin (100 $\mu$M in DMSO) to a concentration of 1 $\mu$M, 30 s prior to the addition of the $^{86}Rb^+$ solution. The parasite-induced and $Ca^{2+}$-activated $^{86}Rb^+$ fluxes were calculated by subtracting the residual $^{86}Rb^+$ influx measured in uninfected cells (in the absence of ionomycin) from that measured in either malaria-infected cells or uninfected cells pre-treated with ionomycin at each inhibitor concentration. Influx rates are expressed as a percentage of those measured in the absence of piperine and quinine. The results are from a single experiment and are representative of those obtained in three similar experiments.

cells it should be more sensitive to quinine-inhibition than the channel in uninfected cells. It is clear from Fig. 2b that the parasite-induced pathway was much less sensitive to inhibition by quinine than was the Gardos channel of uninfected cells.

The quinine-inhibition results reported here indicate that the high residual $^{86}Rb^+$ uptake in infected cells cannot be accounted for simply in terms of the activation of a functionally normal endogenous pathway. If the enhanced flux was via the Gardos channel, the pathway was apparently modified in such a way as to reduce its sensitivity to quinine-inhibition. An alternative explanation for the high residual flux is that it was via a parasite-derived transport system that is absent from the uninfected cell. It has been suggested that such systems are responsible for the enhanced transport of a wide variety of substrates across the infected erythrocyte membrane [13,14] however their nature and identify are yet to be clearly established.

K.K. was supported by an Oxford Nuffield Medical Fellowship. B.C.E. and R.A.P. were supported by the Medical Research Council and the Wellcome Trust.

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