Increased choline transport in erythrocytes from mice infected with the malaria parasite *Plasmodium vinckei vinckei*

Henry M. STAINES*† and Kiaran KIRK*[1]

*Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra ACT 0200, Australia, and †University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Parasitized erythrocytes from mice infected with the murine malaria parasite *Plasmodium vinckei vinckei* showed a marked increase in the rate of influx of choline compared with erythrocytes from uninfected mice. In contrast, uninfected erythrocytes from *P. vinckei*-infected animals transported choline at the same rate as those from uninfected mice. The increased influx of choline into parasitized cells was via two discrete routes. One was a saturable pathway with a K_m similar to that of the choline carrier of normal erythrocytes but a V_{max} approx. 20-fold higher than that observed in uninfected cells. The other was a non-saturable pathway inhibited by furosemide. At choline concentrations within the normal physiological plasma concentration range, the former pathway contributed approx. two-thirds and the latter approx. one-third of the influx of choline into parasitized cells. The characteristics of the furosemide-sensitive pathway were similar to those of a broad-specificity pathway that is induced in human erythrocytes infected *in vitro* with *Plasmodium falciparum*. The results of this study rule out the possibility that the induced transport pathway of *P. falciparum*-infected erythrocytes is an artifact arising *in vitro* from the long-term culture of parasitized cells and provide evidence that this pathway makes a significant contribution to the uptake of choline into the parasitized cells of malaria-infected animals.

INTRODUCTION

The growth and asexual reproduction of the intracellular malaria parasite within the erythrocytes of its vertebrate host entails the synthesis of new membranes. The phospholipid content of the malaria-infected erythrocyte increases by up to 500 % during the course of parasite development, with the bulk (85 %) of the new lipid being either phosphatidylcholine or phosphatidylethanolamine [1]. The synthesis of phosphatidylcholine *de novo* within the intracellular parasite is reliant on the uptake of choline from the external medium and it has been demonstrated that in monkey erythrocytes infected with the malaria parasite *Plasmodium knowlesi* the transport of choline into the parasitized erythrocyte is a rate-limiting step for phosphatidylcholine biosynthesis [2].

Choline is transported into normal mammalian erythrocytes via a well-characterized saturable carrier system (reviewed in [3]). Several previous studies have investigated the transport of choline into malaria-infected erythrocytes, with somewhat differing results. In parasitized erythrocytes from monkeys infected with *P. knowlesi* there is a marked increase in the rate of influx of choline via a pathway that has the same K_m and pharmacological characteristics as the endogenous choline transporter, but a V_{max} some 10-fold higher than that in uninfected erythrocytes [4]. In contrast, in human erythrocytes infected *in vitro* with *P. falciparum* there is a new choline uptake route that differs both kinetically and pharmacologically from the host cell transporter [5]. This pathway has the characteristics of a broad-specificity pore or channel that, although showing a marked preference for anions and electroneutral solutes, has a substantial permeability both to organic cations such as choline and to inorganic cations such as K^+ and Rb^+ [6]. The *P. falciparum*-induced pathway is non-saturable (up to millimolar concentrations of substrate), is inhibited by a variety of anion transport blockers (including furosemide) and transports univalent cations at a rate that varies with the nature of the anion(s) present in the suspending medium [7].

The broad-specificity, furosemide-sensitive pathway induced by *P. falciparum* in parasitized human erythrocytes has been proposed to serve a variety of physiological roles in the malaria-infected cell [7–11]. These include the uptake of essential nutrients, the efflux of metabolic wastes and the volume-regulatory release of intracellular solutes. From a chemotherapeutic viewpoint the pathway holds significant interest, both as a target in its own right and as a route for the selective delivery of cytotoxic drugs into parasitized cells [8–10]. However, although the pathway has been characterized in some detail in cells infected by the malaria parasite *in vitro*, it remains to be established whether the same pathway is present in erythrocytes infected *in vivo*. In the study of choline transport into *P. knowlesi*-infected monkey erythrocytes it was concluded that there was no evidence for choline transport via a pathway of this sort [4] and it has been suggested that the enhanced non-saturable influx of choline into human erythrocytes infected with *P. falciparum* might be an artifact, perhaps arising *in vitro* from the long-term culture of parasitized cells [12].

In this study we have characterized the transport of choline into erythrocytes from mice infected with *P. vinckei vinckei*. The aims of this study were twofold. The first was to establish whether the furosemide-sensitive pathway is present in erythrocytes from *P. vinckei*-infected mice and, if so, its degree of similarity with the pathway described in human erythrocytes infected *in vitro* with *P. falciparum*. The second was to assess the relative contributions of the different transport pathways present in malaria-infected erythrocytes to the uptake of choline from the external medium.

EXPERIMENTAL

Chemicals

[^{14}C]Choline chloride and [^{14}C]taurine were from Amersham Corp. $^{86}Rb^+$ was from DuPont–NEN. Unlabelled choline chloride (recrystallized three times) and furosemide were obtained

[1] To whom correspondence should be addressed (e-mail kiaran.kirk@anu.edu.au).

from Sigma. Furosemide was added to cell suspensions as a stock solution in DMSO.

Malaria-infected erythrocytes

Male CBA/H mice (6–8 weeks old) were injected intraperitoneally with 10^6 erythrocytes containing the malaria parasite *Plasmodium vinckei* ssp. *vinckei* at approx. 30 % parasitaemia. At 6–8 days after infection, when the parasitaemia was in the range 6–50 %, the mice were anaesthetized with ether and the blood was collected via cardiac puncture into sodium heparin (20 i.u./ml of blood). Blood from age-matched uninfected mice was obtained by the same method. Infected mice yielded between 0.1 and 0.4 ml of erythrocytes, whereas uninfected mice typically yielded 0.5 ml of erythrocytes. Where necessary, blood from different mice was pooled to give the number of cells required for each experiment. The blood (containing 0.5–1 ml of cells) was placed on ice then passed over a cellulose CF-11 column at 4 °C to remove peripheral-blood leucocytes. The column was formed in a 20 ml glass syringe, filled with an 80 % (w/v) suspension of cellulose in RPMI 1640 to give a final column height of 15–20 mm. The cells were eluted from the column by washing through with RPMI 1640, then washed four times by centrifugation and resuspension in Hepes-buffered saline (155 mM NaCl/10 mM Hepes/5 mM glucose, pH 7.4). For the first two washes the medium was supplemented with 0.5 % (w/v) BSA, as this was found to minimize haemolysis during the washing procedure.

In some experiments parasitized cells were concentrated by centrifugation on a phosphate-buffered 62.5 % (v/v) Percoll solution (pH 7.4; 300 m-osm/kg of water) as described elsewhere [13]. The method yielded suspensions of more than 60 % parasitaemia (mostly trophozoite/schizont stage) in the top layer and less than 5 % parasitaemia (mostly ring stage) in the lower layer.

Cell counts were made with an improved Neubauer counting chamber. Parasitaemia was estimated from methanol-fixed Giemsa-stained smears.

Choline influx measurements

Estimates of the unidirectional influx rates for choline were made from the uptake of [^{14}C]choline at 37 °C by using methods similar to those described previously [10]. Influx commenced with the addition of cells suspended in Hepes-buffered saline to microcentrifuge tubes containing [^{14}C]choline and unlabelled substrate (at appropriate concentrations), giving a final activity of 0.3 μCi/ml, a final cell concentration of $(1–4) \times 10^8$ cells/ml and a final sample volume of 1.0 ml. At appropriate times, 0.2 ml aliquots of the suspension were transferred to microcentrifuge tubes containing 0.3 ml of dibutylphthalate; the flux was terminated by centrifugation (10000 ***g*** for 20 s) of the tubes to sediment the cells beneath the oil. The aqueous supernatant solution was removed by aspiration and the radioactivity remaining on the walls of the tube was removed by rinsing the tubes four times with water. The dibutylphthalate was aspirated, then the cell pellet was lysed with 0.1 % (v/v) Triton X-100 (0.5 ml) and deproteinized by the addition of 5 % (w/v) trichloroacetic acid (0.5 ml), followed by centrifugation (10000 ***g*** for 10 min). Radioactivity was measured with a β-scintillation counter.

The extracellular space in the cell pellet was estimated in each experiment from the [^{14}C]choline present in pellets derived from samples taken within a few seconds of combining the cells with radiolabel.

Initial experiments involved full uptake time courses in which influx was terminated over a range of predetermined time intervals (see Figure 1, for example). In later experiments choline influx rates were estimated from the amount of radiolabel that accumulated within fixed times that fell within the initial linear portion of the uptake time course (7 min for infected cells and 15 min for uninfected cells).

In experiments in which the Cl^- in the suspending medium was replaced with a range of alternative univalent anions, the cells were washed four times, then resuspended in medium containing 155 mM NaBr, NaI, $NaNO_3$ or NaSCN, together with 10 mM Hepes and 5 mM glucose (pH adjusted to 7.4).

Influx of taurine and K^+ ($^{86}Rb^+$)

The influx of choline was compared with that of the sulphonic amino acid taurine and the inorganic cation K^+ (with the use of $^{86}Rb^+$ as a convenient tracer). Experiments were performed as described for choline. Taurine influx was measured by using [^{14}C]taurine (0.3 μCi/ml) with a final extracellular taurine concentration of 1 mM. K^+ influx was estimated from the uptake of $^{86}Rb^+$ (1 μCi/ml) from medium containing 5 mM KCl + 150 mM NaCl in place of 155 mM NaCl.

For the K^+ ($^{86}Rb^+$) influx experiments the cells were pretreated (for 10 min) with 0.1 mM ouabain to inhibit the erythrocyte Na^+/K^+ pump, 0.01 mM bumetanide to inhibit the $NaKCl_2$ co-transporter and 0.01 mM nitrendipine to inhibit the Ca^{2+}-activated K^+ channel [14].

RESULTS

Increased choline influx into *P. vinckei*-infected erythrocytes

Normal mammalian plasma choline levels are in the range 10–40 μM [15]. When erythrocytes from *P. vinckei*-infected mice were exposed to choline concentrations within this range, the rate of choline influx was substantially higher than that into erythrocytes from normal, uninfected animals. This is illustrated in Figure 1 for cells suspended in medium containing 20 μM choline.

Blood from infected animals had parasitaemias within the range 6–50 % at the time of harvesting. To establish whether the increased rate of choline transport in erythrocytes from *P. vinckei*-infected animals was a feature of the parasitized cells alone or of both parasitized and non-parasitized cells from infected mice, the erythrocytes from infected mice were sub-

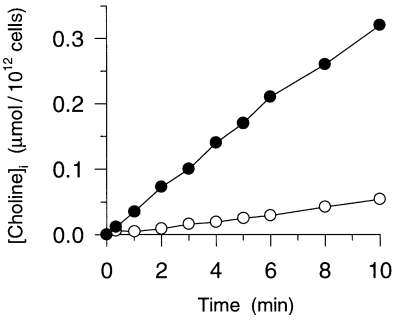

Figure 1 Time courses for the influx of choline into erythrocytes from uninfected mice (○) and mice infected with *P. vinckei vinckei* (●) (46% parasitaemia)

The extracellular choline concentration was 20 μM. The results are averaged from a single experiment performed in triplicate with blood pooled from two infected and two uninfected mice. Standard deviations fall within the symbols.

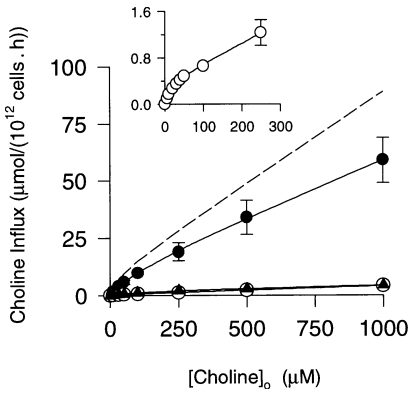

Figure 2 Concentration-dependence of the influx of choline into erythrocytes from uninfected mice (○, main panel and inset) and into predominantly infected erythrocytes (●) (top layer from centrifugation on Percoll; 60–73% parasitaemia) and predominantly uninfected erythrocytes (▲) (bottom layer from centrifugation on Percoll) from mice infected with *P. vinckei vinckei*

For each cell population the results were fitted to the equation $V_0 = [V_{max}[S]_o/(K_m + [S]_o)] + k_d[S]_o$ as described in the text (solid lines). The broken line is that fitted to results for erythrocytes infected at 100% parasitaemia (calculated by subtracting the contribution of the uninfected cells to the influx measured in the high-parasitaemia cell suspensions). The inset shows a more detailed view of the concentration-dependence of choline influx into erythrocytes from uninfected mice. The results are averaged from three separate experiments, each on erythrocytes from different mice. Error bars denote S.E.M.; where not shown, they lie within the symbols.

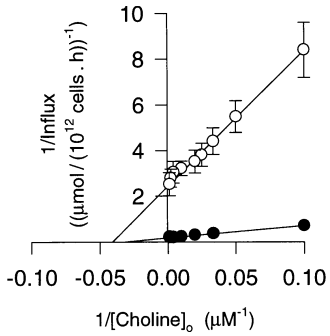

Figure 3 Lineweaver–Burk (i.e. double-reciprocal) plot for the saturable component of the influx of choline into erythrocytes from uninfected mice (○) ($n = 4$) and into *P. vinckei*-infected mouse erythrocytes (●) (100% parasitaemia; $n = 10$)

The rate of influx of choline into parasitized erythrocytes (i.e. erythrocytes with a parasitaemia of 100%) at each choline concentration was calculated by subtracting the contribution of the uninfected cells to the influx measured in infected cell suspensions with parasitaemias in the range 35–73%. The saturable component of choline influx was calculated as described in the text. Error bars denote S.E.M.; where not shown, they lie within the symbols.

fractionated by centrifugation on a Percoll layer (see the Experimental section). In erythrocytes from the top layer (60–73% parasitaemia; predominantly trophozoite/schizont-stage parasites) the rate of choline influx was significantly higher ($P < 0.02$; paired t test) than that measured in erythrocytes from uninfected mice over the full range of choline concentrations tested (10–1000 μM; Figure 2). In contrast the rate of influx of choline into cells from the bottom layer (less than 5% parasitaemia; ring-stage parasites) was not significantly different from that measured

Table 1 Kinetic parameters for the influx of choline into erythrocytes from uninfected mice (RBC) and parasitized erythrocytes (corrected to 100% parasitaemia) (pRBC) from mice infected with *P. vinckei vinckei*

Results are given as means ± S.E.M.

	K_m (μM)	V_{max} (μmol/(10^{12} cells.h))	k_d (μmol/(10^{12} cells.h.μM))
RBC ($n = 4$)	25 ± 2	0.45 ± 0.07	0.0037 ± 0.0007
pRBC ($n = 10$)	42 ± 6	9.5 ± 1.7	0.072 ± 0.007
P (unpaired t test)	> 0.1	< 0.01	< 0.001

in cells from uninfected animals ($P > 0.4$) at any of the choline concentrations tested.

Figure 2 (main panel) shows the rate of choline influx into the erythrocytes from normal, uninfected mice, and into parasitized erythrocytes (top layer from Percoll gradient) and predominantly non-parasitized erythrocytes (bottom layer from Percoll gradient) from infected animals over the full range of choline concentrations. The broken line corresponds to the rate of influx calculated for pure parasitized cells (i.e. cells at 100% parasitaemia).

The inset to Figure 2 shows an expanded view of the concentration-dependence of the rate of influx of choline into erythrocytes from uninfected animals.

In each case the concentration-dependence results could be resolved into two components: a saturable component, conforming to Michaelis–Menten kinetics, and an additional non-saturable component. The dependence of the initial influx rate (V_0) on the extracellular choline concentration ($[S]_o$) could therefore be fitted to an equation of the form:

$$V_0 = [V_{max}[S]_o/(K_m + [S]_o)] + k_d[S]_o$$

where V_{max} and K_m have their customary meanings and k_d is a 'diffusion' constant.

In practice, for each experiment, k_d was estimated directly by fitting a straight line (of slope k_d) to the concentration-dependence results obtained in the higher concentration range (100–1000 μM). The fitted line was used to estimate the contribution of the linear (non-saturable) component to the flux measured at each choline concentration. This contribution was subtracted from the total flux and the remaining (saturable) component was then graphed in the form of a Lineweaver–Burk (double-reciprocal) plot to yield estimates (from the axes intercepts) of K_m and V_{max} for the saturable component of choline influx in the different erythrocyte populations.

Figure 3 shows Lineweaver–Burk plots for the saturable transport component for erythrocytes from uninfected animals and for *P. vinckei*-infected erythrocytes (corrected to 100% parasitaemia). The kinetic parameters for these two cell populations are given in Table 1. In *P. vinckei*-infected erythrocytes the non-saturable influx component (k_d) and the V_{max} for the saturable component were both increased by approx. 20-fold relative to those in uninfected erythrocytes. In contrast, the K_m for the saturable transport component was not significantly altered.

Inhibition of the non-saturable component of choline influx into *P. vinckei*-infected erythrocytes by furosemide

The observation of a marked increase in the non-saturable component of choline influx into *P. vinckei*-infected mouse

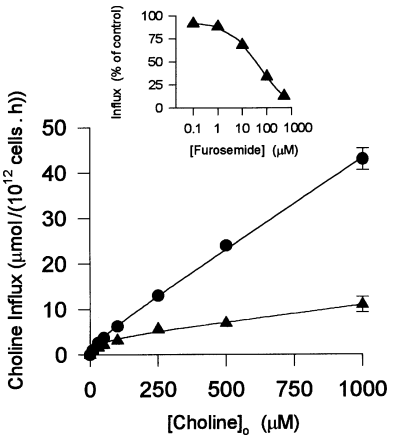

Figure 4 Concentration-dependence of the influx of choline into erythrocytes from *P. vinckei*-infected mice measured in the presence (▲) and the absence (●) of 0.2 mM furosemide

The inset shows the dose–response curve for the effect of furosemide on the influx of choline into erythrocytes from *P. vinckei*-infected mice, measured at an extracellular choline concentration of 10 mM to minimize the uptake of radiolabel via the saturable transport pathway. The IC_{50} (i.e. the concentration of furosemide required to decrease the rate of choline influx to 50% of the value in the absence of inhibitor) was 36 ± 4 μM. The data are averaged from three separate experiments, each on erythrocytes from different mice. Error bars denote S.E.M.; where not shown, they lie within the symbols.

Table 2 Kinetic parameters for the influx of choline into *P. vinckei*-infected mouse erythrocytes suspended in the presence and the absence of 0.2 mM furosemide

The results are averaged from three separate experiments and are given as means ± S.E.M.

	K_m (μM)	V_{max} (μmol/(10^{12} cells . h))	k_d (μmol/(10^{12} cells . h . μM))
Control	33 ± 8	2.8 ± 0.4	0.0410 ± 0.0025
+ Furosemide	39 ± 4	3.1 ± 0.5	0.0090 ± 0.0009
P (paired *t* test)	> 0.5	> 0.6	< 0.001

erythrocytes is similar to the situation in human erythrocytes infected *in vitro* with *P. falciparum* [5]. The non-saturable uptake of choline into *P. falciparum*-infected human erythrocytes is inhibited by the anion transport blocker furosemide [6]. We therefore tested the effect of this reagent on the transport of choline into erythrocytes from *P. vinckei*-infected mice.

Figure 4 (main panel) shows the rate of influx of choline into erythrocytes from *P. vinckei*-infected mice, measured over a range of choline concentrations, in the presence and the absence of 0.2 mM furosemide. The kinetic parameters (derived as outlined above) are given in Table 2. The furosemide caused a marked (80%) inhibition of the large, parasite-induced non-saturable influx component (k_d) but left the saturable component unaffected.

Furosemide (0.2 mM) had no effect on either the saturable or non-saturable components of choline influx into erythrocytes from uninfected mice (results not shown).

The inset to Figure 4 shows a dose–response curve for the effect of furosemide on the non-saturable component of the influx of choline into erythrocytes from *P. vinckei*-infected mice. In these experiments the influx of [^{14}C]choline was measured in cells suspended (at the time of exposure to radiolabel) in medium containing 10 mM choline to saturate and thereby minimize the uptake of radiolabelled solute via the saturable choline transporter. The IC_{50} for inhibition (i.e. the concentration of furosemide required to decrease influx to 50% of the value in the absence of inhibitor) was 36 ± 4 μM.

Table 3 Anion dependence of the rate of influx of choline via the furosemide-sensitive pathway of erythrocytes from *P. vinckei*-infected mice

The suspending medium contained 10 mM Hepes, 5 mM glucose and 10 mM choline chloride, together with 155 mM NaCl, NaBr, NaI, $NaNO_3$ or NaSCN. The furosemide-sensitive influx was obtained by subtracting the flux measured in the presence of 0.2 mM furosemide from that measured in its absence. The furosemide-sensitive fluxes are shown as a percentage of those measured in medium containing Cl^- as the sole permeant anion (control). The results are means ± S.E.M. for three experiments, each on erythrocytes from different donor mice.

Anion	Furosemide-sensitive influx (% of control)
Cl^- (control)	100
Br^-	149 ± 13
I^-	175 ± 15
NO_3^-	192 ± 6
SCN^-	227 ± 15

Anion dependence of the non-saturable component of parasite-induced choline influx

A distinguishing feature of the transport of choline and other univalent cations via the parasite-induced pathway of *P. falciparum*-infected erythrocytes is a marked dependence on the nature of the anion present in the suspending medium; the flux of cations via the pathway increases substantially on replacement of Cl^- with NO_3^-, SCN^-, I^- or Br^- [7]. As summarized in Table 3, the furosemide-sensitive influx of choline into erythrocytes, from *P. vinckei*-infected mice, washed and resuspended in medium containing a range of different anions, varied markedly with the anion in the suspending medium, increasing in the order $Cl^- < Br^- < I^- < NO_3^- < SCN^-$.

Furosemide-sensitive transport of other solutes

The furosemide-sensitive pathway of *P. falciparum*-infected human erythrocytes is permeable to a diverse range of low-molecular-mass solutes [6]. To test whether the same might be true of the pathway responsible for the furosemide-sensitive uptake of choline into *P. vinckei*-infected erythrocytes, we measured the influx of two structurally unrelated solutes: the inorganic cation $^{86}Rb^+$ and the electroneutral sulphonic amino acid taurine.

As illustrated in Figure 5(A), taurine permeated erythrocytes from uninfected mice at a low but significant rate that was unaffected by the presence of 0.2 mM furosemide. Erythrocytes from mice infected with *P. vinckei* showed a significantly elevated rate of taurine influx. Furosemide (0.2 mM) decreased the rate of influx in cells from infected animals to close to that seen in normal uninfected erythrocytes.

With K^+ ($^{86}Rb^+$) (Figure 5B) there was, under the conditions of the experiment, a sizable influx into cells from uninfected animals. This influx showed a slight sensitivity to furosemide, with 0.2 mM furosemide causing a small decrease in the rate of $^{86}Rb^+$ influx. Under the same conditions, erythrocytes from infected animals showed an increased rate of $^{86}Rb^+$ influx. As with taurine, the addition of furosemide decreased the influx of

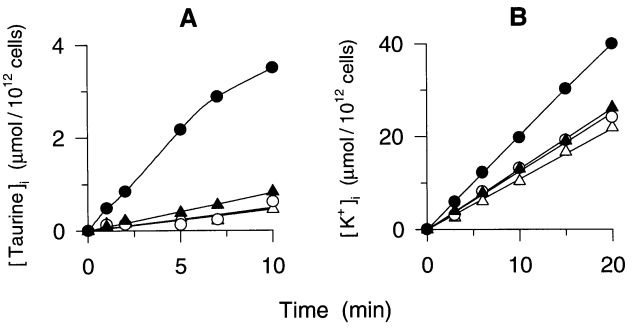

Figure 5 Time courses for the influx of the electroneutral sulphonic amino acid taurine (A) and the inorganic cation K^+ ($^{86}Rb^+$) (B) into erythrocytes from uninfected mice (○, △) and mice infected with *P. vinckei vinckei* (16% parasitaemia) (●, ▲), suspended in the absence (○, ●) and the presence (△, ▲) of 0.2 mM furosemide

The extracellular taurine and K^+ concentrations were 1 and 5 mM respectively. $^{86}Rb^+$ uptake was measured in the presence of ouabain (0.1 mM), bumetanide (0.01 mM) and nitrendipine (0.01 mM) to inhibit the endogenous erythrocyte Na^+/K^+ pump, the $NaKCl_2$ co-transporter and the Ca^{2+}-activated K^+ channel respectively. The results are representative of those obtained in two separate experiments, each on erythrocytes from different donor mice.

$^{86}Rb^+$ into infected cells to a similar level to that seen in erythrocytes from uninfected animals.

In a single paired experiment in which the magnitudes of the furosemide-sensitive influx of taurine and choline were compared, they were found to be similar. In two separate experiments the furosemide-sensitive influx of taurine and choline was found in each case to be approximately twice that of Rb^+ (results not shown).

DISCUSSION

Erythrocytes from mice infected with *P. vinckei vinckei* showed a marked increase in the rate of influx of choline. The increase was restricted to the parasitized cells from the infected animal and was mediated by two discrete pathways: a saturable (and furosemide-insensitive) pathway with a similar K_m to the endogenous choline transporter of normal mouse erythrocytes, and an additional, non-saturable transport route that was inhibited by furosemide. In infected cells exposed to choline at a concentration within the normal physiological range (20 μM), the enhanced saturable transport route contributed approx. two-thirds of the influx of choline, whereas the non-saturable, furosemide-sensitive pathway contributed approx. one-third of the influx.

The saturable transport pathway

The finding that choline was taken up into *P. vinckei*-infected mouse erythrocytes via a saturable pathway with a K_m very similar to that of the endogenous erythrocyte choline transporter, but a V_{max} some 20-fold greater than that in uninfected cells, is similar to the findings of Ancelin et al. [4] with erythrocytes from *P. knowlesi*-infected monkeys. In that study the V_{max} for choline transport into parasitized erythrocytes was approx. 10-fold higher than that into uninfected erythrocytes, with the pathway responsible having the same K_m and the same sensitivity to a number of inhibitors as the endogenous transporter [4].

Similar results were obtained in a recent study of the transport of putrescine, a polyamine, into *P. knowlesi*-infected monkey erythrocytes [16]. Putrescine was taken up into infected erythrocytes by a saturable pathway with a similar K_m to the putrescine transporter of normal erythrocytes but a V_{max} some 3-fold that seen in uninfected cells. It is possible that choline and putrescine share the same carrier (both are cations at physiological pH) and that the increase in the rate of transport of both substrates can be attributed to the increased activity of a single class of carrier. However, this has not been tested directly.

The origin of the increase in saturable choline transport observed in the present study (and previously) remains unclear. Perhaps the most likely explanation is that the increased flux is via transporters endogenous to the host erythrocyte, although the possibility of its being via a parasite-encoded transporter with the same affinity for choline as the mammalian system cannot be excluded. The enhanced saturable uptake component might be due, at least in part, to the parasites' invading preferentially the younger erythrocytes in the circulation as these have been shown (in humans) to have a much higher rate of choline transport than older erythrocytes [17]. Alternatively (or additionally) the intracellular parasite might actually increase the activity of the transporters in the host erythrocyte membrane, perhaps via a modification of the proteins themselves or of the lipid environment in which the transporters operate [18,19].

The non-saturable pathway

The observation of a substantial influx of choline into *P. vinckei*-infected erythrocytes via a non-saturable pathway that, at higher choline concentrations, becomes the major route for choline entry, is similar to the situation for human erythrocytes infected *in vitro* with *P. falciparum* [5,6]. It differs somewhat from the results obtained by Ancelin et al. [4,12], who reported that in parasitized erythrocytes from *P. knowlesi*-infected monkeys there was no evidence for an increase in the influx of choline via any route other than the saturable carrier.

The pathway responsible for the increased non-saturable influx of choline into parasitized mouse erythrocytes shows characteristics very similar to those of the new permeation pathway induced by the intracellular parasite in *P. falciparum*-infected human erythrocytes [8–11]. Like the pathway induced in human erythrocytes [6], that in mouse erythrocytes is inhibited by furosemide, and the rate of influx of choline via this pathway shows a similar anion dependence ($SCN^- > NO_3^- > I^- > Br^- > Cl^-$) to that seen for the influx of cations into *P. falciparum*-infected human erythrocytes (SCN^-, $NO_3^- > I^-$, $Br^- > Cl^-$) [7]. The induced pathway of *P. falciparum*-infected human erythrocytes is permeable to a diverse range of small organic and inorganic solutes, including taurine and Rb^+ ([6], and H. A. Horner and K. Kirk, unpublished work). The observation of a furosemide-sensitive influx of these two solutes, taurine and Rb^+, into *P. vinckei*-infected mouse erythrocytes (Figure 5) is consistent with the same being true of the pathway that mediates the enhanced non-saturable influx of choline into these cells.

Previous studies have indicated the presence in malaria-infected mouse erythrocytes of a pathway with at least some of the characteristics of the induced pathway of *P. falciparum*-infected human erythrocytes. Homewood and Neame [20] reported the uptake into parasitized (but not non-parasitized) erythrocytes from *P. berghei*-infected mice of L-glucose, a compound that is transported into *P. falciparum*-infected erythrocytes via the furosemide-sensitive pathway [13]. Gati et al. [21] described the entry into erythrocytes from *P. yoelii*-infected mice of both D-adenosine and L-adenosine, via a parasite-induced pathway that was inhibited by furosemide with an IC_{50} of 15–17 μM, compared with the IC_{50} of 36 μM found here. These values are slightly higher than those reported for the inhibition of the induced permeation pathway of human erythrocytes infected *in vitro* with *P. falciparum* (1–5 μM) [6,22,23]. It has been suggested that the

selectivity characteristics of the transport pathways induced by the intracellular malaria parasite vary with the host and parasite species [11]. However, the extent of this variation remains to be established.

Conclusions

Parasitized erythrocytes from mice infected with *P. vinckei vinckei* showed a marked increase in the rate of influx of choline. When exposed to extracellular choline concentrations within the normal physiological range, approx. two-thirds of the influx was via a saturable transporter that operated with the same K_m as the choline carrier of normal, uninfected erythrocytes, but with a V_{max} approx. 20-fold higher than that seen in uninfected cells. Under the same conditions one-third of the influx was via a non-saturable pathway showing similar characteristics to the pathway induced in human erythrocytes infected *in vitro* with *P. falciparum*. This finding rules out the possibility that the induced transport pathway of *P. falciparum*-infected human erythrocytes is an artifact generated *in vitro* [12]. Furthermore it raises the possibility that *P. vinckei*-infected mice offer a suitable model in which to test the chemotherapeutic opportunities presented by the induced permeation pathway, either as a target in its own right or as a route for selectively targeting cytotoxic compounds into the parasitized cell.

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