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RED CELL AMINO ACID TRANSPORT

EVIDENCE FOR THE PRESENCE OF SYSTEM *Gly* IN GUINEA PIG RETICULOCYTES

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Guinea pig reticulocytes are shown to possess an Na^+-dependent glycine transporter which also requires Cl^- for activity. Glycine transport by this route is saturable (apparent K_m 98 μM; V_{max} 24 μmol/g Hb per h) and inhibited by sarcosine. The properties of this carrier closely resemble those of System *Gly* previously demonstrated in pigeon and human erythrocytes. In contrast, no System *Gly* activity was detected in mature guinea pig erythrocytes.

A number of functionally distinct amino acid transport systems have been described in mammalian erythrocytes [1,2]. Three of these are Na^+-dependent (*ASC*, *Gly* and *Glu*), while one (*Gly*) also requires Cl^- for activity. An interesting feature of erythrocyte amino acid transport is that the distribution of amino acid carriers in the red blood cells of different species is highly variable. For example, System *Gly* is a high-affinity transporter that is selective for glycine and sarcosine and is found in both avian and human erythrocytes [3–5], but is not present in mature rabbit or sheep red blood cells [4,6]. In contrast, Na^+-dependent glycine transport has been observed in both rabbit and sheep reticulocytes. Thus, Winter and Christensen [6] described two saturable routes for Na^+-dependent glycine uptake by rabbit reticulocytes, one with an apparent K_m of approx. 40 μM, the other with an apparent K_m of 6 mM. Benderoff et al. [7] reported the presence of electrogenic glycine transport in sheep reticulocytes. These observations suggested to us that System *Gly* might be present in reticulocytes from many, if not all, mammalian species, with differences in erythrocyte System *Gly* activity arising, at least in part, during reticulocyte maturation. We report here an investigation of glycine transport in guinea pig reticulocytes and erythrocytes. Our results demonstrate that mature guinea pig erythrocytes lack System *Gly* activity, whereas reticulocytes from this species are shown to possess a high-affinity Na^+/Cl^--dependent glycine transporter which is inhibited by sarcosine.

To obtain reticulocytes, adult Dunkin Hartley guinea pigs were made anaemic by heart puncture under ether anaesthesia, removing a total of 30–50 ml blood over a period of 5 consecutive days. Reticulocyte-rich blood (typically 30–50% reticulocytes [8]) was collected on the third day of rest following the bleeding sequence. Alternatively, animals were injected subcutaneously with 2.5% (w/v) phenylhydrazine-HCl in 0.9% (w/v) saline (1 ml/1200 g body weight) for 5 consecutive days. The percentage of reticulocytes on the third day of rest was similar to that induced by phlebotomy, and the two preparations exhibited comparable

glycine transport characteristics.

Fig. 1 shows the results of a representative experiment in which the uptake of glycine (0.1 mM extracellular concentration, 37 °C) by reticulocyte-enriched blood (37% reticulocytes) from an anaemic guinea pig was measured in NaCl, $NaMeSO_4$, $KMeSO_4$ and KCl media and compared with glycine transport by mature erythrocytes from a normal adult guinea pig. K^+ and $MeSO_4^-$ were employed as Na^+ and Cl^+ substitutes, respectively. Glycine transport in normal NaCl medium was considerably more rapid in reticulocytes than in mature erythrocytes. This difference in glycine uptake between the two cell preparations was abolished when reticulocyte glycine transport was measured in the absence of Na^+ ($KMeSO_4$ and KCl media), demonstrating the presence of Na^+-dependent glycine transport activity in guinea pig reticulocytes. The data presented in Fig 1 show that a large fraction of Na^+-dependent glycine uptake by reticulocytes was also Cl^--dependent. Equilibration values (1 h incubation) for glycine uptake by the reticulocyte-rich cell preparation were 1.06, 0.62, 0.34 and 0.28 μmol/g Hb in NaCl, $NaMeSO_4$, $KMeSO_4$ and KCl media, respectively. Na^+-dependent uptake therefore accounted for 0.75 μmol glycine transported/g Hb. Of this, 59% was Cl^--dependent. In contrast, neither Na^+ nor Cl^- substitution significantly affected equilibration values for glycine uptake by mature guinea pig erythrocytes (0.24, 0.24, 0.23 and 0.26 μmol/g Hb after 1 h in NaCl, $NaMeSO_4$, $KMeSO_4$ and KCl media, respectively). Thus, mature guinea pig erythrocytes do not exhibit measurable Na^+/Cl^--dependent glycine transport activity.

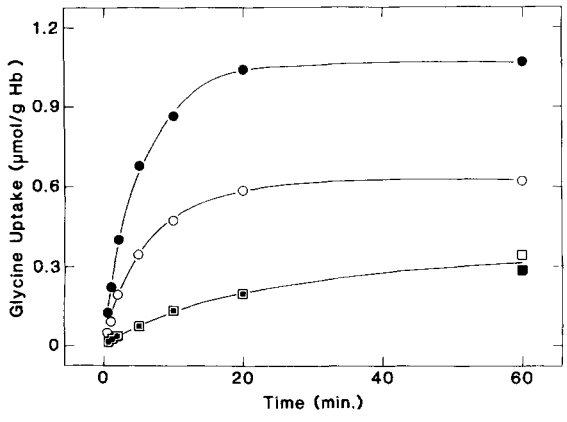

Fig. 1. Time-course of glycine uptake by guinea pig reticulocytes and erythrocytes. [1-^{14}C]Glycine (Radiochemical Centre, Amersham, Bucks, U.K.) uptake (0.1 mM, 37 °C) was measured by the *n*-dibutylphthalate centrifugation technique described in detail by Young and Ellory [13]. Briefly, equal volumes of prewarmed radioactive amino acid in different isotonic media containing either NaCl, $NaMeSO_4$, $KMeSO_4$ or KCl (150 mM), 15 mM Mops (morpholinepropane sulphonate, pH 7.5 at 37 °C) and 5 mM glucose were mixed with either reticulocyte-rich or mature erythrocyte cell suspensions (haematocrit approx. 20%) equilibrated in the respective media. Aliquots (0.2 ml) were sampled at predetermined time intervals and uptake was stopped by transferring the cell suspension into a 1.5 ml microcentrifuge tube containing 0.8 ml of ice-cold incubation medium layered on top of 0.5 ml of ice-cold *n*-dibutylphthalate. The tube was immediately centrifuged (15 s, 15000 × *g*). Both the aqueous and *n*-dibutylphthalate phases were removed by aspiration and the inside of the tube was carefully wiped with absorbent tissue paper. The cell pellet was then processed for liquid scintillation counting by adding 0.5 ml of 0.1% (v/v) Triton X-100 in water followed by 0.5 ml 5% (w/v) trichloroacetic acid. The precipitate was removed by centrifugation (2 min, 15000 × *g*) and the protein-free supernatant counted for radioactivity with appropriate quench correction. The trapped extracellular space following centrifugation through *n*-dibutylphthalate was measured by processing cell samples that had been mixed with radioactive amino acid at 0 °C and immediately centrifuged. Uptake values were calculated after subtraction of these blanks. Values are means of duplicate estimates. The percentage of reticulocytes in the reticulocyte-rich blood was 37% as judged by supravital staining with Brilliant Cresyl Blue [8]. Symbols: reticulocyte glycine uptake in NaCl (●), $NaMeSO_4$ (○), $KMeSO_4$ (□) and KCl (■) media. Glycine uptake by mature red blood cells in all four media was found to co-plot with reticulocyte uptake in $KMeSO_4$ and KCl media.

To characterize further the glycine transport in guinea pig reticulocytes we measured the initial rate of glycine uptake as a function of extracellular glycine concentration over the concentration range 0.02–0.30 mM in NaCl, $NaMeSO_4$, $KMeSO_4$ and KCl media. The results presented in Fig. 2 confirm that substitution of K^+ for Na^+ results in a substantial decrease in glycine uptake (94% at 0.03 mM and 88% at 0.3 mM), the residual glycine transport in the absence of Na^+ showing a linear concentration dependence. Two components of Na^+-dependent glycine uptake are apparent, one of which is saturable and requires Cl^- for activity. The magnitude of Na^+/Cl^--dependent glycine uptake was found to be 73% and 47% of the total uptake at 0.03 and 0.3 mM extracellular glycine, respectively. Na^+/Cl^--dependent glycine trans-

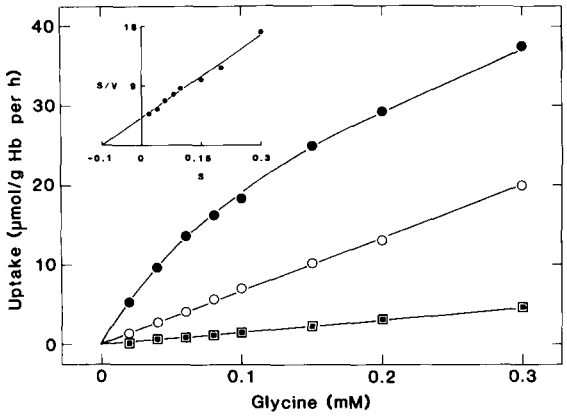

Fig. 2. Concentration dependence of glycine uptake by guinea pig reticulocytes. Initial rates of [1-^{14}C]glycine uptake (1 min incubation) were measured at 37 °C in NaCl (●), $NaMeSO_4$ (○), $KMeSO_4$ (□) and KCl (■) media as described in the legend to Fig. 1. The insert is a Hanes (S/V vs. S) plot of the Na^+/Cl^--dependent (NaCl − $NaMeSO_4$ uptake) glycine transport. Values are means of duplicate estimates. The reticulocyte count of the cell preparation was 48%.

port (NaCl − $NaMeSO_4$ uptake) conformed to simple Michaelis-Menten kinetics, giving a V_{max} of 24.4 μmol/g Hb per h. The apparent K_m value of 98 μM is similar to that reported previously for System *Gly* from human and avian erythrocytes [3–5] and corresponds well with the apparent K_m of high-affinity glycine transport in rabbit reticulocytes [6]. Data presented in Table I demonstrate that Na^+/Cl^--dependent glycine uptake (0.1 mM extracellular concentration) by guinea pig reticulocytes was inhibited by sarcosine (86% inhibition at 2 mM sarcosine). In contrast, Na^+-dependent, Cl^--independent glycine transport (difference between $NaMeSO_4$ and either $KMeSO_4$ or KCl uptake rates) by guinea pig reticulocytes was not inhibited by sarcosine and exhibited a linear concentration dependence. This low-affinity uptake is likely to be mediated by System *ASC* as has been shown to occur in both avian and human erythrocytes [3,4,9,10]. Guinea pig reticulocytes (but not mature red blood cells) were found to exhibit Na^+-dependent transport of L-alanine, a System *ASC* substrate (data not shown).

We conclude from these experiments that guinea pig reticulocytes possess an Na^+/Cl^--dependent glycine transporter (System *Gly*) with properties similar to that described in mature human and avian erythrocytes [3–5]. This carrier is presumably equivalent to the high-affinity glycine transporter found in rabbit reticulocytes [6] and the carrier responsible for the electrogenic glycine transport observed in sheep reticulocytes [7]. In this context it is interesting to note that reticulocytes have a high glycine requirement for haem formation. System *Gly* was found to be functionally absent from mature guinea pig erythrocytes. Our data therefore support the view that species differences in erythrocyte glycine transport arise during reticulocyte maturation. The finding that in vitro maturation of sheep reticulocytes is associated with regression of Na^+-dependent glycine and L-alanine transport activity is consistent with this conclusion [7,11]. We envisage that reticulocyte maturation in some species is associated with the total loss of System *Gly* activity, whereas erythrocytes from other species retain a functional *Gly* transporter. Even the oldest circulating human erythrocytes exhibit measurable Na^+/Cl^--dependent glycine transport activity [12].

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TABLE I

EFFECT OF SARCOSINE ON GLYCINE UPTAKE BY GUINEA PIG RETICULOCYTES

Initial rates (30 s incubation) of glycine uptake (0.1 mM extracellular amino acid, 37 °C) were measured in NaCl, $NaMeSO_4$, $KMeSO_4$ and KCl media in the presence and absence of sarcosine (2 mM extracellular amino acid). The reticulocyte count of the cell preparation was 37%. Transport rates are expressed as μmol/g Hb per h. Values are means (±S.E.) of triplicate estimates.

	Incubation medium			
	NaCl	$NaMeSO_4$	$KMeSO_4$	KCl
Control	13.81 ± 0.45	7.06 ± 0.15	1.38 ± 0.06	1.38 ± 0.02
Sarcosine	8.13 ± 0.22	6.37 ± 0.19	1.25 ± 0.05	1.21 ± 0.04

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