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AMINO ACID TRANSPORT PROPERTIES OF ERYTHROCYTES FROM NORMAL NEWBORN LAMBS AND LAMBS WITH AN INHERITED DEFECT IN AMINO ACID TRANSPORT

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Summary

An amino acid transport defect which occurs in the erythrocytes of adult sheep is also present in foetal erythrocytes from newborn lambs which have inherited the lesion. The transport defect in erythrocytes from adult sheep is associated with high intracellular levels of ornithine and lysine and a markedly diminished GSH concentration. Although the lesion in foetal cells also results in the accumulation of ornithine and lysine, the intracellular GSH concentration is only moderately diminished.

Sheep fall into two distinct types according to the amino acid transport properties of their erythrocytes [1,2]. Some sheep have cells which possess a stereospecific transport system (the cysteine system) which has a high affinity for neutral amino acids of intermediate size such as cysteine. The dibasic amino acids ornithine and lysine have a lower, but still significant affinity for the system. Other sheep lack this transport system and their erythrocytes are characterized by having a markedly diminished glutathione (GSH) concentration, a reduced potential life span and an increased susceptibility to oxidative stress [3,4]. These cells also have high concentrations of a number of amino acids, notably ornithine and lysine [5], hence the designation Ly+ and Ly— for transport-deficient and normal animals, respectively [6]. The present paper presents data on amino acid transport and intracellular GSH and amino acid concentrations in foetal erythrocytes from newborn lambs.

Heparinized blood samples were collected from Finnish Landrace cross-bred lambs 1—3 days after birth. Erythrocytes were washed 3 times with 20 vols. of

Abbreviation: DTNB, 5,5′-dithiobis-(2-nitrobenzoate).

ice-cold 0.92% (w/v) NaCl and then fractionated by centrifugation [7,8] to yield a cell population containing higher than 90% foetal cells (i.e., cells containing foetal haemoglobin) as judged by the acid elution technique of Moore et al. [9]. Initial amino acid uptake rates (0.2 mM) were determined at 37°C as previously described [1] and amino acid concentrations were assayed after sulphosalicylic acid extraction using a Locarte amino acid analyser. Erythrocyte GSH concentrations were determined using 5,5′-dithiobis-(2-nitrobenzoate) (DTNB) [10] and alloxan [11] as chromogens.

There was a 17-fold difference in L-alanine uptake rate between foetal cells from Ly+ and Ly− animals (Table I). L-Lysine showed a smaller, but still significant difference in transport rate. In contrast, the uptake of L-phenylalanine, which is not a substrate for the cysteine system, was the same in the two types of animal. For comparison, the legend to Table I includes previously published data for erythrocytes from adult sheep [1]. The transport rates in foetal erythrocytes are faster than in adult cells (1.1—2.5-fold for Ly− animals and 2.2—4.3-fold for Ly+ animals), but both the selectivity towards different amino acids and the permeability differences between the two types of animal were very similar in foetal and adult erythrocytes. Experiments performed in the absence of Na^+ demonstrated that, as in adult erythrocytes, L-alanine uptake by foetal cells was not Na^+ dependent.

Table II summarises the dibasic amino acid concentrations of foetal erythrocytes from Ly+ and Ly− animals. Cells from Ly+, but not Ly− sheep showed the characteristic high intracellular concentrations of ornithine and lysine found in adult Ly+ erythrocytes [5]. Interestingly, one of the Ly+ lambs also had an inherited arginase deficiency [12], resulting in the accumulation of arginine rather than ornithine. The intracellular GSH concentrations of foetal erythrocytes from Ly+ and Ly− lambs were compared using the non-specific thiol chromogen DTNB [10]. Cells from Ly+ animals had a singificantly lower DTNB-GSH concentration than erythrocytes from Ly− lambs (1.57 ± 0.13

TABLE I

AMINO ACID TRANSPORT IN FOETAL ERYTHROCYTES FROM Ly^+ and Ly^- NEWBORN LAMBS

The amino acid concentration was 0.2 mM and incubation times (10 min for L-alanine and 30 min for L-lysine and L-phenylalanine) were chosen to give initial uptake rates [1]. Values are means ± S.E. (range). For comparison with these data the initial rates of L-alanine, L-lysine and L-phenylalanine uptake (0.2 mM) by erythrocytes from adult sheep are 154 ± 6.6, 15.0 ± 1.0 and 27.6 ± 2.2 μmol/l cells per h respectively for Ly− animals and 2.1 ± 0.4, 4.6 ± 1.0 and 29.0 ± 3.6 μmol/l cells per h, respectively, for Ly+ sheep (mean ± S.E., n = 4) [1].

	Uptake (μmol/l cells per h)			Number of animals
	L-Alanine	L-Lysine	L-Phenyl-alanine	
Ly−	173.3 ± 15.5 (139.2—228.1)	30.0 ± 4.3 (22.5—46.3)	67.5 ± 4.7 (55.3—79.3)	5
Ly+	9.1 ± 1.9 (4.6—13.6)	10.2 ± 2.1 (5.6 —15.1)	67.3 ± 3.6 (59.3—75.1)	4
P *	<0.001	<0.01	n.s.	

* Student's t-test (n.s., not significant).

TABLE II

DIBASIC AMINO ACID CONCENTRATIONS OF FOETAL ERYTHROCYTES FROM Ly— AND Ly+ NEWBORN LAMBS

Values are means ± S.E. (range). The ornithine and lysine concentrations of erythrocytes from adult sheep are 0.2 ± 0.08 and 0.28 ± 0.11 mmol/l cells, respectively, for Ly— animals and 5.3 ± 0.8 and 7.8 ± 1.0 mmol/l cells respectively for Ly+ animals (mean ± S.E., n = 6) [5].

	Concentration (mmol/l cells)			Number of animals
	Ornithine	Lysine	Arginine	
Ly—	0.22 ± 0.07 (0—0.39)	0.35 ± 0.10 (0—0.60)	0.17 ± 0.11 (0—0.52)	5
Ly+	5.73 ± 2.06 (2.65—9.63)	7.74 ± 2.94 (3.90—13.5)	0.16 ± 0.16 (0—0.48)	3
P *	<0.02	<0.02	n.s.	
Ly^+, arginase-deficient	0.29	4.75	5.01	1

* Student's t-test (n.s., not significant).

(5) (1.30—2.05) and 2.18 ± 0.11 (5) (1.84—2.53) mmol/l cells, respectively (mean ± S.E. (n) (range)) $P < 0.01$ by Student's t-test). Control experiments using the GSH-specific chromagen alloxan [11] established that 75% of the DTNB-reactive thiol in foetal cells from Ly— lambs was GSH compared with 85% in Ly+ animals.

These results demonstrate that amino acid transport in foetal sheep erythrocytes is significantly faster than in adult sheep erythrocytes. Quantitatively similar differences for amino acid transport have been found in the pig [13]. However, although the rate of amino acid uptake is increased in foetal sheep erythrocytes, the pattern of selectivity towards different amino acids is similar in foetal and adult cells, and the amino acid transport lesion described in Ly+ adult sheep erythrocytes is also found in the foetal cells of lambs which have inherited this lesion. This situation is quite different from the other transport systems so far investigated in foetal sheep erythrocytes. Thus, glucose and nucleoside transport are considerably faster in foetal cells [14,15], and in the case of nucleoside transport, foetal erythrocytes showed a rapid uptake rate even when taken from lambs with a genetically determined variant which lacks the nucleoside transport system as an adult [16,17]. Another system present in the foetal erythrocyte and absent in the adult is the Ca-stimulated K-channel [18]. In the case of the Na pump, potentially LK (low potassium phenotype) and HK (high potassium phenotype) animals show high transport rates in their foetal cells [8,19]. Thus, amino acid transport is the only system so far studied which is similar in the foetal and adult situation.

Two consequences of the amino acid transport defect in adult sheep erythrocytes are increased intracellular concentrations of a number of amino acids, particularly ornithine and lysine, and decreased levels of GSH. From Table II it is apparent that transport-deficient foetal cells also accumulate high concentrations of ornithine and lysine. In the case of an arginase-deficient lamb, arginine was accumulated rather than ornithine. A similar effect of arginase deficiency has also been observed in adult sheep [20]. Thus, the ornithine which is found in Ly+ cells is derived from arginine. The origin of these amino acids remains to

be elucidated; one possibility is that they arise from protein degradation during cell maturation (see for example ref. 21).

A major role of amino acid transport in the adult sheep erythrocyte is to ensure adequate intracellular levels of cysteine for GSH biosynthesis. It is, therefore, interesting that the GSH concentrations of Ly− and Ly+ foetal cells differed only by 28%, compared with the 3-fold difference seen in adult erythrocytes (see also ref. 6). In contrast, another lesion, which results in a diminished activity of γ-glutamyl cysteine synthetase, the first enzyme of GSH biosynthesis, causes a considerable reduction in the GSH concentration of foetal sheep erythrocytes [6,22]. The reasons for these differences remain to be resolved since nothing is known about GSH turnover in the foetal cell situation, and the comparison of foetal and adult erythrocytes is complicated by considerable differences in cell life span [23] and the plasma concentrations of some amino acids [24,25].

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