

Comparative Erythrocyte Metabolism In Marsupials And Monotremes

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Abstract

Concentrations of ATP and DPG, activities of 10 enzymes and the glycolytic rates were measured in the erythrocytes of 11 species of marsupials and two species of monotremes. Mean DPG concentrations were greater in the erythrocytes of marsupials than those of eutherian mammals. The opposite is true of ATP. Significant findings from the results of enzyme activities were: high activity of hexokinase (7.39 ± 0.82 EU/g Hb) in the short-beaked echidna, pyruvate kinase (37.49 ± 1.0 EU/g Hb) in bridled nailtail wallaby and glucose-6-phosphate dehydrogenase (G6PD; 41.66 ± 1.24 EU/g Hb) in black-striped wallaby. About 6- to 7-fold difference in the activity of G6PD levels between the two species of wombats was confirmed. Glucose phosphate isomerase activity was also shown to be twice as high in the red cells of the common wombat compared with those of the southern hairy nosed wombat. There were wide variations in the glycolytic rate among the species examined.

Keywords: Erythrocytes; marsupials; monotremes; wallaby; kangaroo; wombat; echidna; platypus

Introduction

The mammalian red blood cell (RBC) is an ideal subject for investigation in several areas of molecular biology, physiology and genetics. RBC enzymes are useful as genetic markers and in the diagnosis of (i) hereditary haemolytic anaemia, (ii) genetic non-haematological diseases (e.g. galactosaemia) and (iii) nutritional deficiencies (e.g. riboflavin). Comparative studies have continued to unravel a close association between red cell metabolism and oxygen affinity of haemoglobin (Isaaks and Harknes, 1983).

However, almost all of these studies have been carried out either on human RBC or on those from eutherian (placental) mammals. There is a great paucity of information on RBC metabolism in marsupials and/or monotremes. Comparative studies on RBC may provide models for pathophysiology processes in human and/or veterinary medicine, prompting us to undertake the present studies.

Materials and Methods

Animals included in the present study were six species of wallabies: allied rock wallaby (*Petrogale assimilis*), black-striped wallaby (*Macropus dorsalis*), bridled nailtail wallaby (*Onychogalea fraenata*), proserpine rock wallaby (*Petrogale persephone*), swamp wallaby (*Wallabia bicolor*), and tammar wallaby (*Macropus eugenii*); two species of kangaroos: eastern grey kangaroo (*Macropus giganteus*), and red kangaroo (*Macropus rufus*); two species of wombats: common wombat (*Vombatus ursinus*) and southern hairy nosed wombat (*Lasiorhinus latifrons*) and finally the northern brown bandicoot (*Isoodon macrourus*); short-beaked echidna (*Tachyglossus aculeatus*) and platypus (*Ornithorhynchus anatinus*). The sources of animals were: Taronga Zoo, Sydney, NSW, CSIRO Division of Wildlife and Ecology, ACT, Queensland National Parks and Wildlife Macropod collection at Pallarenda Townsville QLD and Texas in Southern Queensland.

Blood was collected from the lateral caudal vein from the kangaroos and wallabies, by cardiac puncture from the bandicoots and echidna, from the bill sinus of the platypus, and from the brachial vein in the wombats. Blood-sample volumes ranged from 3 to 10 ml depending on the size of the animal. The blood was collected into tubes containing lithium heparin. The samples were placed on ice and transported by air to Armidale and were received within 24 hr. Packed cell volume (PCV) and haemoglobin (Hb) concentrations were determined by standard laboratory procedures. Blood samples that were to be assayed for ATP and DPG were precipitated immediately after sampling, with an equal volume of ice-cold 8% (w/v) trichloroacetic acid, and were frozen until analysis. Samples were centrifuged prior to assay, and the supernatant used to determine ATP and DPG content according to relevant Sigma Technical bulletins.

Haemolysates were prepared in order to assay the following enzymes: hexokinase (HK), glucosephosphate isomerase (GPI); phosphfructokinase (PFK); aldolase, glyceraldehyde-3- phosphate dehydrogenase (GAPD); phosphoglycerate kinase

(PGK); pyruvate kinase (PK); lactate dehydrogenase (LDH); glucose-6-phosphate dehydrogenase (G6PD); and 6-phosphogluconate dehydrogenase (6PGD). Enzyme assays were carried out at 37°C. Since recent studies by Grigg *et al.* (1992a,b) have shown that the body temperature of active echidnas and platypus in the field is close to 32°C, enzyme assays in the monotremes were therefore made at 32°C. Changes in absorbance were recorded using a Beckman DU-64 spectrophotometer. The methods used in the preparation of the haemolysates and the assay systems were those described by Beutler (1984).

Lactate production in RBC, incubated with different substrates, was measured using the methods described by Bethlenfalvay *et al.* (1984) with some minor modifications. Washed RBC were suspended in a "suspension" buffer (120 mM NaCl, 30 mM Na₂HPO₄, 5 mM KC1, 1.2 mM MgCl₂, and 50 mM Tris, pH 7.4) to give a final haematocrit of -15%. RBC suspensions were incubated with one of the four substrates: 10 mM glucose, 5 mM adenosine, 5 mM inosine and 10 pL of autologous plasma per ml RBC, in a water bath at 37°C (or 32°C for the echidna). Lactate was measured in samples taken at 0, 1 and 2 hr intervals by the method of Godwin *et al.* (1983).

Results and Discussion

The results shown in Table I indicate that RBC of the eastern grey kangaroo had very low concentrations of ATP (0.069 µmol/ml RBC) and those of red kangaroo the highest (0.53 µmol/ml RBC). Other species had values that were within this range. Published values of ATP concentration in the RBC of marsupials range from being undetectable in the echidna (Kim *et al.*, 1981) and in the long-nosed potoroo (Agar *et al.*, 1989) to 1.75 µmol/ml RBC in the parma wallaby (Agar *et al.*, 1986).

DPG concentrations in marsupials are comparatively higher than eutherian mammals (Isaacks *et al.*, 1984; Agar *et al.*, 1989; Agar and Godwin, 1991; Agar and Spencer, 1993a,b). This was again evident in this study (Table 1). The reason for the relatively higher levels of DPG in the RBC of marsupials is not known. Marsupials utilize DPG as a haemoglobin modulator in a similar fashion to eutherian mammals (Bland and Holland, 1977). Farber and Tenney (1971) have shown that the pouch of the American marsupial, *Virginia opossum*, has relatively low concentrations of O₂ and high concentrations of CO₂. Whether high levels of DPG in marsupials are in some way related to their early life in the possibly hypoxic environment of the pouch is not known.

Table I. Levels of ATP and DPG in the erythrocytes of various species of marsupials*

Metabolite	Bridled nailtail wallaby (n = 6)	Prosperpine rock wallaby (n = 4)	Allied rock wallaby (n = 5)	Black- striped wallaby (n = 6)	Eastern Grey kangaroo (n = 14)	Red kangaroo (n = 7)
ATP (µ mol/ml RBC)	0.41 ± 0.05	0.17 ± 0.02	0.21 ± 0.02	0.15 ± 0.01	0.069 ± 0.01	0.53 ± 0.06
DPG (µmol/ml RBC)	3.78 ± 0.14	4.66 ± 0.52	3.92 ± 0.40	5.92 ± 0.13	6.26 ± 0.24	6.47 ± 0.57

*Mean ± SE; n = number of animals

Data presented in Table 2 indicate that there were wide variations in the activities of some enzymes, while the differences in the activities of other enzymes were not pronounced. Activity of HK, one of the rate limiting enzymes, varied from as low as 0.46 (in red kangaroo) to as high as 7.39 (in echidna), while the range for aldolase was only from 0.40 (in northern brown bandicoot) to 1.19 (in bridled nailtail wallaby). Activity of another rate limiting enzymes, PK also varied greatly from 2.41 (in the common wombat) to 37.49 (in the bridled nailtail wallaby), as while there was not so much variation in the activity of 6PGD. Although interspecies comparison of erythrocyte energetics may not only be difficult to interpret but may also be of little physiological significance, such information may, however, provide an insight into the evolutionary and/or environmental impact on the survival of red cells. Data on G6PD values in the two species of wombat throw some light on this point.

The observation of a 6- to 7-fold difference in G6PD activity between the two wombat species, common wombat and southern hairy nosed wombat, reported by Agar *et al.* (1989) was confirmed in this study (Table 2). A further observation of a greater than 2-fold difference in GPI activity was also made. Partial characterization of these two enzymes showed that, although pH optima were similar, K_m values differed significantly: the K_m value for G6PD for glucose 6-phosphate in the common wombat was 70 μM , and 130 μM in the southern hairy nosed wombat. The K_m values of GPI for fructose-6-phosphate were 83 and 170 μM in the two wombats, respectively. These differences in K_m values indicate differences in substrate affinities of the enzymes in the two species. Differences in G6PD activity are found in wallaroos living in two different populations; it is higher in wallaroos living in the arid zones of Central Australia compared with the populations in the wet eastern coastal regions (Richardson and Czuppon, 1969). The two types of wombats also live in two different environments; the common wombat lives in temperate areas on the east coast of Australia, and the southern hairy nosed wombat in the semi-arid areas of South Australia. Whether these differences have some evolutionary and/or adaptive significance is not known and warrants further investigation.

Wide variations in lactate production rates of the RBC from the different species were noted (Table 3). In general the rates of lactate production were greater when the cells were incubated with autologous plasma. However, the exception was the bridled nailtail wallaby (Table 3). Very high lactate production rates with autologous plasma have previously been seen in the red legged pademelon (Agar and Spencer, 1993a) and the spectacled hare-wallaby (Agar and Spencer, 1993b). RBC of eutherian mammals have also been shown to produce large quantities of lactate when incubated with their own plasma (Kim, 1983). As would be expected, glucose was also a good substrate for energy production in all species. The two nucleosides used, adenosine and inosine, were comparatively poor substrates. Whether low levels of lactate production by the red cells of marsupials and monotremes are a reflection of poor permeability of these substrates through the cell membrane or deficiencies of the enzymes associated with the breakdown of these molecules was not ascertained.

In conclusion, we have found a wide range of activities of 10 enzymes involved in the hexose monophosphate shunt and glycolysis, as well as a wide range of concentrations of the key "high energy metabolite", ATP, and the oxygen-affinity regulatory metabolite DPG, in the RBCs from 13 species of Australian native animals.

Table 2. Enzyme activities in the erythrocytes of various species of marsupials and monotremes*

Enzyme	brown bandicoot (n = 2)	Common wombat (n = 2)	Southern hairy wombat (n = 2)	Swamp wallaby (n = 4)	Tammar wallaby (n = 4)	Bridled nailtail wallaby (n = 4)	Allied rock wallaby (n = 4)	Enzyme activity (E.U./g Hb)	Prosperpine rock wallaby (n = 4)	Black- striped wallaby (n = 4)	Eastern grey kangaroo (n = 3)	Red kangaroo (n = 3)	Echidna (n = 6)	Platypus (n = 5)
HK	1.20, 3.04	1.45, 1.19	0.41, 0.54	0.73 ± 0.07	0.31 ± 0.03	1.53 ± 0.25	1.43 ± 0.08	0.95 ± 0.23	3.31 ± 0.06	1.70 ± 0.06	0.46 ± 0.05	7.39 ± 0.82	2.71 ± 0.53	
GPI	39.93, 43.48	36.7, 43.48	95.21, 91.30	22.40 ± 0.66	23.69 ± 1.02	163.33 ± 21.21	24.15 ± 1.32	55.25 ± 4.47	68.02 ± 3.48	33.24 ± 1.65	23.30 ± 3.26	15.38 ± 1.89	21.09 ± 3.22	
PFK	133, 3.62	132, 1.20	0.82, 1.45	0.98 ± 0.19	0.68 ± 0.04	2.95 ± 0.40	2.23 ± 0.33	1.81 ± 0.23	3.08 ± 0.19	0.64 ± 0.02	1.25 ± 0.37	3.77 ± 0.57	1.21 ± 0.27	
Ald	0.33, 0.47	0.94, 0.80	1.15	0.69 ± 0.15	1.11 ± 0.07	1.19 ± 0.10	0.94 ± 0.13	0.75 ± 0.03	0.69 ± 0.07	0.48 ± 0.09	—	0.54 ± 0.10	0.55 ± 0.04	
GAPD	77.02, 82.70	49.80, 25.44	44.44, 24.46	55.53 ± 7.06	68.25 ± 9.05	56.29 ± 9.48	28.40 ± 3.28	66.52 ± 7.84	102.28 ± 9.36	39.05 ± 3.47	28.80 ± 4.11	45.59 ± 3.99	68.18 ± 13.9	
PGK	78.06, 75.51	160.66, 75.30	130.10	81.38 ± 5.84	70.40 ± 4.73	159.88 ± 24.37	104.55 ± 6.22	93.59 ± 3.47	162.18 ± 12.61	63.98 ± 4.12	64.52 ± 4.16	141.88 ± 3.99	48.48 ± 7.27	
PK	6.54, 2.47	16.01, 5.0	2.37, 0.50	7.91 ± 1.44	6.70 ± 0.29	37.49 ± 1.0	21.42 ± 0.57	10.02 ± 2.54	33.58 ± 0.69	6.51 ± 0.98	1.16 ± 0.10	3.89 ± 0.81	11.66 ± 2.07	
LDH	44.25, 76.79	142.69, 99.40	93.79, 89.14	40.84 ± 5.80	61.31 ± 6.82	76.62 ± 7.33	48.43 ± 1.59	131.74 ± 1.96	243.53 ± 3.18	52.47 ± 6.84	21.64 ± 0.20	103.96 ± 5.00	165.62 ± 8.32	
G6PD	11.42, 17.06	3.15, 5.03	29.72, 21.07	8.68 ± 2.62	7.27 ± 0.35	19.59 ± 0.66	18.81 ± 1.69	17.41 ± 0.79	41.66 ± 1.24	23.08 ± 1.53	5.78 ± 0.22	27.17 ± 3.24	23.54 ± 2.14	
6PGD	5.66, 7.36	1.75, 1.98	1.75, 1.30	2.24 ± 0.18	1.72 ± 0.15	2.69 ± 0.16	2.41 ± 0.54	2.69 ± 0.11	4.04 ± 0.10	1.54 ± 0.20	3.25 ± 0.24	5.99 ± 0.81	6.17 ± 0.49	

*Mean ± SE; n = number of animals.

Table 3. Lactate production rates in the erythrocytes of various species of marsupials and monotremes*

Substrate	Echidna (n = 6)	Lactate (mmol/ml RBC/hr)								Red kangaroo (n = 3)
		Northern brown bandicoot (n = 2)	Bridled nailtail wallaby (n = 4)	Prosperpine rock wallaby (n = 4)	Allied rock wallaby (n = 4)	Tammar wallaby (n = 4)	Black- striped wallaby (n = 4)	Eastern grey kangaroo (n = 3)	Red kangaroo (n = 3)	
Glucose	2.18 ± 0.41	5.91 ± 2.17	3.88 ± 0.87	2.90 ± 0.65	2.54 ± 0.21	3.96 ± 0.16	3.73 ± 0.81	3.48 ± 1.94	5.47 ± 0.62	
Adenosine	1.85 ± 0.33	6.95 ± 0.88	—	0.93 ± 0.44	2.20 ± 0.62	1.88 ± 0.15	1.23 ± 0.58	2.17 ± 0.36	1.83 ± 0.06	
Inosine	0.71 ± 0.09	3.95 ± 1.01	0.45 ± 0.15	0.43 ± 0.20	1.12 ± 0.26	1.77 ± 0.21	0.78 ± 0.34	1.16 ± 0.53	1.84 ± 0.16	
Plasma	2.52 ± 0.24	5.86 ± 0.52	0.64 ± 0.21	15.64 ± 3.36	10.42 ± 1.40	11.61 ± 1.94	16.61 ± 3.33	15.21 ± 2.05	12.98 ± 0.14	

*Mean ± SE; n = number of animals.

References

- Agar N. S. and Godwin I. R. (1991) Red cell metabolism in the brown antechinus, the bilby and the rufous hare-wallaby. *Austr. J. Zool.* **39**, 681-687.
- Agar N. S., Godwin I. R., Suzuki M., Hablethwaite J., Roberts J. and Hume I. D. (1986) Comparative red blood cell metabolism in three wallaby species, *Macropus eugenii*, *Macropus parma*, and *Thylogale thetis* (Macropodidae: Marsupialia). *Comp. Biochem. Physiol.* **85A**, 297-299.
- Agar N. S., Godwin I. R., Wells C. A. and Wallis I. R. (1989) Erythrocyte metabolism in potoroid, macropodid, and vombatid marsupials. In *The Red Cell: Seventh Ann. Arbor Conference* (Edited by Brewer G. J.), pp. 3-21. Alan R. Liss, New York.
- Agar N. S. and Spencer P. B. S. (1993a) Levels of glycolytic enzymes and metabolites in the red blood cells of the red-legged Pademelon, *Thylogale stigmatica* (Macropodidae: Marsupialia). *Comp. Biochem. Physiol.* **105B**, 199-202.
- Agar N. S. and Spencer P. B. S. (1993b) Hematology and erythrocyte biochemistry of spectacled hare-wallaby, *Lagorchestes conspicillatus* (Macropodidae: Marsupialia). *Int. J. Hemat.* **58**, 9-13.
- Bethlenfalvay N. C., Lim J. E. and Waldrup T. (1984) Studies on the energy metabolism of opossum (*Didelphis virginiana*) erythrocytes. I. Utilisation of carbohydrates and purine nucleosides. *J. Cell. Physiol.* **120**, 69-74.
- Beutler E. (1984) *Red Cell Metabolism. A Manual of Biochemical Methods*. Grune and Stratton, New York.
- Bland D. K. and Holland R. A. B. (1977) Oxygen affinity and 2,3-diphosphoglycerate in blood of Australian marsupials of differing body size. *Respir. Physiol.* **31**, 279-290.
- Board P. G. and Agar N. S. (1983) Adenosine triphosphate metabolism in erythrocytes from the Australian echidna. *IRCS Med. Sci.* **11**, 530.
- Farber J. P. and Tenney S. M. (1971) The pouch gas of the virginia opossum (*Didelphis virginiana*). *Respir. Physiol.* **11**, 335-345.
- Godwin I. R., Agar N. S. and Roberts J. (1983) Measurement of erythrocyte ATP, DPG, glucose and lactate with a Cobas-Bio centrifugal analyser. *Clin. Chem.* **29**, 1855.

- Grigg G. C., Augee M. L. and Beard L. A. (1992a) Thermal relations of free-living echidnas during activity and in hibernation in a cold climate. In *Platypus and Echidnas* (Edited by Augee M. L.), pp. 160-173. Royal Zoology Society NSW.
- Grigg G., Beard L., Grant T. and Angee M. (1992b) Body temperature and diurnal activity patterns in the platypus (*Ornithorhynchus anatinus*) during winter. *Austr. J. Zool.* **40**, 135-142.
- Isaacks R., Nicol S., Sallis J., Zeidler R. and Kim H. D. . (1984) Erythrocyte phosphates and hemoglobin function in monotremes and some marsupials. *Am. J. Physiol.* **246**, R236-R241.
- Isaacks R. E. and Harkness D. R. (1983) Erythrocyte organic phosphates and hemoglobin function in domestic mammals. In *Red Blood Cells of Domestic Mammals* (Edited by Agar N. S. and Board P. G.), pp. 315-337. Elsevier, Amsterdam.
- Kim H. D. (1983) Postnatal changes in energy metabolism of mammalian red blood cells. In *Red Blood Cells of Domestic Mammals* (Edited by Agar N. S. and Board P. G.), pp. 339-355. Elsevier, Amsterdam.
- Kim H. D., Zeidler R. B., Sallis J. D., Nichol S. C. and Isaacks R. E. (1981) Adenosine triphosphate deficient erythrocytes of the egg-laying mammal, Echidna (*Tachyglossus aculeatus*). *Science*, **213**, 1517-1519.
- Richardson B. J. and Czuppon A. B. (1969) Geographical variation in quantitative red blood cell glucose-6-phosphate dehydrogenase in the euro (*Macropus robustus*; Marsupialia). *Austr. J. Sci.* **32**, 106-107.