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RED CELLS OF NEWBORN RATS HAVE LOW BISPHOSPHOGLYCEROMUTASE AND HIGH PYRUVATE KINASE ACTIVITIES IN ASSOCIATION WITH LOW 2,3-BISPHOSPHOGLYCERATE

John G. Gilman

Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912, USA

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SUMMARY: 2,3-Bisphosphoglycerate is a physiologically important regulator of red cell oxygen affinity during mammalian development. The rat has no fetal hemoglobin, but the newborn red cell has low 2,3-bisphosphoglycerate and high ATP concentrations, and high oxygen affinity. This report shows that red cell bisphosphoglyceromutase activity increases from near zero in the newborn rat to very high levels by four weeks of age. This increase roughly parallels the increase in red cell 2,3-bisphosphoglycerate concentration. Red cell pyruvate kinase activity declines ten-fold from birth to four weeks of age. This decrease is associated with a changeover in red cell populations from larger to smaller cells. The glycolytic rate is at least 50% higher in newborn than adult rat red cells. The data suggest that high pyruvate kinase activity and glycolytic rate contribute to the high ATP concentration in newborn rat red cells, but that their low 2,3-bisphosphoglycerate concentration is due primarily to low bisphosphoglyceromutase activity.

INTRODUCTION

Of the small molecules known to influence the functioning of hemoglobin, 2,3-bisphosphoglycerate (DPG) plays a particularly important role during mammalian development. Human fetal hemoglobin binds DPG relatively poorly (1). This leads to higher oxygen affinity for fetal red cells than for adult red cells, which aids in the oxygenation of fetal blood. Mammals which lack fetal hemoglobin tend to have low concentrations of DPG and high oxygen affinity for their fetal and newborn red cells. This is the case for the pig (2), rabbit (3), dog (4), rat (5) and other mammals.

The enzymatic mechanism responsible for the low fetal red cell DPG level has been studied in the rabbit (6,7) and dog (8,9). The primary cause appears to be the very high red cell pyruvate kinase activity for the fetus and newborn

Abbreviations: 2,3-Bisphosphoglycerate, DPG; Hemoglobin, Hb.

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compared to the adult. Bisphosphoglyceromutase activity does not differ much between fetal or newborn red cells and adult red cells of the rabbit and dog.

I here report that the low DPG concentration of the newborn rat red cell (5) is associated with both very low bisphosphoglyceromutase activity and high pyruvate kinase activity, and that the transition to adult red cell enzyme activities is associated with a changeover of red cell populations.

METHODS

Rats (Rattus norvegicus) were of the Long-Evans strain (Charles River Breeding Laboratories, Wilmington, MA). Both male and female rats were used, except that only females were used for the determination of adult red cell DPG concentration. Adult rats were between 3 and 9 months of age, with the majority 7-8 months old.

Red cell DPG concentrations were determined by a kinetic-rate enzymic assay, using dilute hemolyzates (10). The DPG concentration data of Fig. 1 are as previously reported in reference 5, except for the 22 day, 28 day, and adult values, which had been determined using perchloric acid extracts. Those values of reference 5 were overestimates due to failure to take into account the water content of the blood in calculating dilution factors for perchloric acid extracts. The kinetic rate determinations do not suffer from that complication, and those data for three to four week old rats and adults reported in reference 11 have been used in Fig. 1.

Bisphosphoglyceromutase and pyruvate kinase activities were measured at 37°C as described by Beutler (12), except that the hemolyzing solution contained 0.05 M Tris at pH 9.6. Alkaline pH is necessary to prevent precipitation of rat hemoglobins. The pH of the assay mixture was measured as 7.80 at 37°C after a run. Enzymes and substrates were from Sigma Chemical Co. (St. Louis, MO) and Boehringer Mannheim (Indianapolis, IN). Enzyme activities are expressed as micromoles substrate converted per minute (IU) per gram of hemoglobin. Hemoglobin concentration of the hemolyzate was determined spectrophotometrically on cyanmethemoglobin (13).

The glycolytic rate was determined at 37°C by incubating red cells at a hematocrit value of 20-25 in 50 mM Hepes buffer (pH 7.4 or 7.25) containing 90 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM KH_2PO_4 and $MgSO_4$, and 7 mM glucose. Samples were taken at 30 and 90 or 120 minutes, and extracted with twice the volume of cold 6% perchloric acid for 20 minutes. Lactate (12) and glucose were determined enzymically, the latter by modifying the procedure for assaying ATP (12).

Size distributions of red cells were found by measuring to the nearest 0.5μ the diameters of about 100 cells of a Wright-stained smear, using a calibrated ocular micrometer.

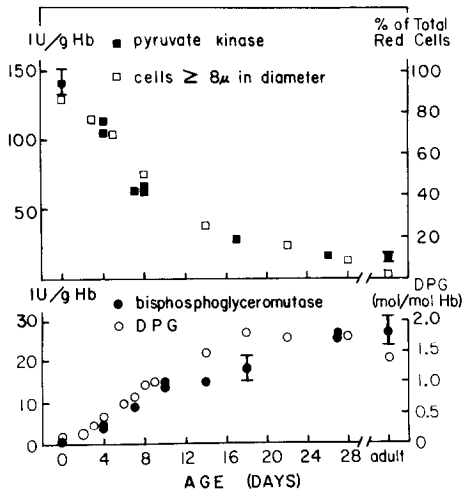

Fig. 1. Red cell pyruvate kinase and bisphosphoglyceromutase activities during rat development after birth. Mean ± S.D. are given for 6 newborn and 7 adult animals for pyruvate kinase, and for 4 animals at day 18 and 12 adults for bisphosphoglyceromutase. The red cell DPG concentrations (previously reported in references 5 and 11) and the distribution of red cell diameters are shown for comparison.

RESULTS AND DISCUSSION

Pyruvate Kinase Activity

The upper portion of Fig. 1 shows that pyruvate kinase activity is very high in rat red cells just after birth. Most of the red cells at this time are very large--greater than 7.5μ in diameter on fixed, Wright-stained blood smears.

Within a few days after birth, red cell pyruvate kinase activity starts to decline. By one week after birth it has fallen to less than half the newborn value. Cells smaller than 8μ in diameter begin to predominate in the circulation by that time.

By about four weeks after birth, the adult value for red cell pyruvate kinase has been attained, and more than 90% of the red cells are smaller than 8μ in diameter. One sees from Fig. 1 that the change in red cell pyruvate kinase activity roughly parallels the change in red cell size distribution. This suggests a changeover from a fetal red cell type to a biochemically distinct and smaller adult type.

Bisphosphoglyceromutase Activity

The lower portion of Fig. 1 shows the very low activity for bisphosphoglyceromutase in rat red cells at birth (data are for seven newborn animals). The sensitivity of the enzyme assay is such that it is difficult to say whether the bisphosphoglyceromutase is absent, or present at a level of activity of about one IU/g Hb. The presence of low but non-zero values of DPG in the red cells of the newborn rat suggests that there is a low level of bisphosphoglyceromutase activity in those cells.

After birth, the red cell bisphosphoglyceromutase activity increases more or less in concert with the increase in DPG concentration. An analogous association of red cell bisphosphoglyceromutase activity and DPG level during development has been observed for the chicken (14).

Glycolytic Rate

In addition to differences in pyruvate kinase and bisphosphoglyceromutase activities, newborn and adult rat red cells differ in their overall rates of glycolysis. Newborn rat red cells have a very high glycolytic rate: At pH 7.4 in 50 mM Hepes buffer, they consumed about 10 μM glucose/(ml cells·hr), while the rate for adult red cells was 5 to 5.5. Under these conditions, the rate of lactate production was 17 μM lactate/(ml cells·hr) for the newborn red cell and 9 to 11 for the adult red cell.

A similar difference between rates of glycolysis for newborn and adult red cells was observed at pH 7.25. The higher glycolytic rate of newborn compared to adult red cells suggests a higher activity for hexokinase and/or phosphofructokinase in newborn red cells, since those two enzymes are the primary rate-controlling enzymes of the glycolytic pathway (15).

CONCLUSION

This report has shown that pyruvate kinase activity of rat red cells falls dramatically in the four weeks following birth, while bisphosphoglyceromutase activity and DPG concentration increase in concert. A decrease in red cell

size parallels these biochemical changes. Work of Valet and colleagues (16) has demonstrated the post-natal turnover of a number of populations of rat red cells which differ in volume. Data on red cell diameters reported here agree with their work, which showed the complete replacement of the red cell populations present at a rat's birth with populations of smaller red cells by 27 days of age (16). Data of this report show distinct enzyme differences in addition to the volume differences for the red cell populations of the newborn compared to those of the adult rat.

Previous reports have shown that high pyruvate kinase activity alone may account for the low DPG level in red cells of the newborn rabbit (6,7) and dog (9). Data of this report suggest that high pyruvate kinase activity in conjunction with a high glycolytic rate may be important in maintaining ATP concentration at the high level seen in newborn rat red cells (5), but that the low DPG level in those cells may be primarily due to very low bisphosphoglyceromutase activity.

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