EFFECT OF ADENOSINE ON GLUCOSE METABOLISM OF RANA RIDIBUNDA ERYTHROCYTES

MARTHA KALOYIANNI, BASILE MICHAELIDIS and KATERINA MOUTOU

Laboratory of Animal Physiology, Department of Zoology, Science School, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece

Accepted 9 December 1992

Summary

The effects of adenosine and inosine on Rana ridibunda erythrocyte metabolism were studied. Adenosine can be metabolized by Rana ridibunda erythrocytes as a carbon source for glycolysis to maintain ATP levels, whereas neither inosine nor glucose could maintain ATP levels. The rate of lactate production was maximal (2.89 μmol ml⁻¹ red cell h⁻¹) in the presence of adenosine together with glucose compared with that in the presence of glucose or adenosine alone. Inosine sustained a similar rate of lactate production as did glucose. The concentrations of phosphorylated hexoses increased during 4 h of incubation in the presence of adenosine either with or without glucose. The presence of sodium iodoacetate accelerated the loss of ATP; furthermore, levels of lactate and pyruvate were significantly reduced in the presence of either adenosine or inosine. Ouabain strongly inhibited ATP and lactate formation in the presence of adenosine, whereas the levels of ATP and lactate were almost unchanged in the presence of inosine. An inhibitor of adenosine transport, dipyridamole, impeded the increases in erythrocyte lactate and ATP concentration in the presence of adenosine but did not modify the effects of inosine.

Introduction

Although the ability of erythrocytes to consume glucose varies greatly, depending upon species and animal age, most mammalian erythrocytes utilize glucose as the major metabolic fuel. Exceptions are pig erythrocytes, which are impermeable to glucose (Kim and McManus, 1971), and cow erythrocytes, which show postnatal changes in enzymes and cofactors (Kim, 1985). In contrast to mammalian erythrocytes, nucleated erythrocytes do not uniformly prefer any particular metabolic substrate. The reports on avian erythrocytes are contradictory: according to Bell (1971) avian erythrocytes do not consume measurable quantities of glucose under basal conditions, whereas Kalomenopoulou and Beis (1990) suggested that Columba livia erythrocytes consume rather low, but measurable, amounts of glucose. Amphibian erythrocytes utilize glucose through the glycolytic pathway to support their energy needs (Kaloyianni-Dimitriades and Beis, 1984).

Key words: adenosine, erythrocytes, glucose metabolism, Rana ridibunda.
Although functioning glycolytic mechanisms are necessary for cell longevity, recent observations indicate that certain non-glycolytic pathways may be equally important. These involve nucleotide metabolism per se rather than reactions which simply generate high-energy compounds for storage in the cell’s adenine nucleotide pool. There has been much interest in erythrocyte metabolism of adenosine in recent years (Plagemann, 1986; Rapoport et al. 1990; Bethlenfalvay et al. 1990; Kim, 1990), possibly because of adenosine’s effectiveness in regenerating the ATP level of stored blood (Overgaard-Hansen, 1957; Scafer and Bartlett, 1962), which was attributed to the direct phosphorylation of adenosine to AMP by adenylate kinase (Lerner and Rubinstein, 1970). Adenosine is metabolized by three distinct pathways catalyzed, respectively, by adenosine kinase, adenosine deaminase and S-adenosylhomocysteine hydrolase, depending on its concentration in the medium (Kim, 1990). It has long been postulated that erythrocytes function as a vehicle for delivering preformed purines to various body organs that are deficient in purine biosynthesis pathways (Lajtha and Vane, 1958); later it was suggested (Plagemann, 1986) that the byproducts of erythrocyte metabolism may serve as precursors of purine biosynthesis in various cells.

Published data on the involvement of purine compounds in erythrocyte metabolism and on their role in substituting for glucose as a metabolic substrate refer only to the non-nucleated erythrocytes of mammals (Jarvis et al. 1986; Kim, 1990; Rapoport et al. 1990; Bethlenfalvay et al. 1990). To our knowledge, only nucleated chicken erythrocytes have been found to depend on adenosine as a carbon source for glycolysis (Espinet et al. 1989). Amphibian red blood cells are typical of red blood cells of non-mammalian vertebrates in that they are nucleated and actively metabolize glucose (Kaloyianni-Dimitriades and Beis, 1984; Kaloyianni and Kalomenopoulou, 1990). The major metabolic determinants, namely the partitioning of glucose or other compounds such as adenine nucleosides through the pathways operating within these erythrocytes, have not yet been elucidated.

In the present study we investigate the metabolic behaviour of nucleated frog erythrocytes suspended in various concentrations of adenosine and inosine. Intact red blood cells (RBCs) were examined in order to approximate more closely their usual physiological state. In addition, the effects of adenosine on some glycolytic intermediates that are considered to be of primary importance for erythrocyte energy metabolism were evaluated. We used metabolic transport inhibitors to examine the relationship between transport and metabolism of glucose and adenosine in frog erythrocytes.

**Materials and methods**

**Animals**

Frogs (*Rana ridibunda*) weighing 50–120g were supplied by a local dealer after having been caught in the vicinity of Thessaloniki. They were kept in containers in fresh water and used a week after arrival.

**Chemicals and enzymes**

The substrates, enzymes, coenzymes and resins were purchased from Sigma Chemical Company (St Louis, USA). All other chemicals were purchased from Serva (Heidelberg, Germany), Mercks or Pharmacia.
Amphibian erythrocyte metabolism

Sampling of red blood cells

Blood samples were obtained from anaesthetised frogs by heart puncture using heparinized syringes; the frogs were subsequently killed by heart removal. Immediately after collection, the blood was centrifuged at 500 g for 10 min and the plasma and surface layer of white cells were removed by aspiration. The erythrocytes were then washed three times with 0.1 mol l⁻¹ NaCl.

Metabolic studies

All metabolic studies were performed with washed red cells drawn from 3–5 animals to give one pool of cells. The washed erythrocytes were subsequently suspended at a haematocrit of 12–18% in buffer (pH 7.4) containing (in mmol l⁻¹): 100 NaCl, 5.9 CaCl₂, 2.4 MgSO₄, 4.2 imidazole, 7.6 glycyglycine, 1.2 KH₂PO₄ and five substrates, in the presence or absence of various inhibitors as indicated in Table 1. Previous results have shown that a pH of 7.4 promotes Rana ridibunda erythrocyte glycolysis (Kaloyianni-Dimitriades, 1983). 2 ml of erythrocyte suspension was gassed with a mixture of 95% O₂ and 5% CO₂ and incubated in 25 ml Erlenmeyer flasks fitted with rubber caps. The flasks were incubated in a shaking water bath at 25°C for 4 h. Samples were removed at the beginning (0 h) and the end (4 h) of incubation and adenosine triphosphate (ATP), glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P), fructose 1,6-diphosphate (FDP), pyruvate and lactate contents were determined.

Preparation of perchloric acid (PCA) extracts for metabolite determination

Red cell metabolites were assayed in neutralized perchloric acid extracts. The extracts were prepared by mixing the packed red cell suspension with the same volume of 70% perchloric acid. After centrifugation at 4000 g for 10 min, the supernatant was neutralized with 0.5 mol l⁻¹ TrisCl in 0.5 mol l⁻¹ KOH. The precipitate of potassium perchlorate was removed by centrifugation at 4000 g for 5 min. The supernatant was used for metabolite determinations.

Determination of intermediates, adenosine triphosphate and lactate

Glucose concentration was estimated by the method of Krebs et al. (1964), pyruvate by the method of Bucher et al. (1963) and ATP by the method of Lamprecht and Trautschold (1963). G-6-P and F-6-P were estimated in the same cuvette by the method of Hohorst (1963), FDP by the method of Bucher and Hohorst (1963) and lactate by the method of Hohorst (1965).

All metabolites were measured spectrophotometrically in a Hitachi recording spectrophotometer which followed the change in optical density at 340 nm caused by the oxidation or reduction of NAD(H) or NADP(H).

Statistical evaluations used Student’s t-tests (P<0.005) applied to unpaired grouped samples.

Results

Table 1 shows the effect of the studied nucleosides and glucose on Rana ridibunda erythrocyte glycolysis. When frog erythrocytes were incubated in the absence of a
metabolic substrate, ATP level declined after 4h of incubation to about 58% of the initial value (3.08±0.04\,\mu\text{mol}\text{ml}^{-1}\text{RBC}). However, a net synthesis of ATP was observed in the presence of adenosine. The maximum net synthesis of ATP that occurred in the presence of adenosine either with or without glucose was at least 40% higher than the ATP content measured in media containing glucose alone (Table 1). Neither inosine nor glucose with inosine was able to maintain ATP at the initial level.

In the substrate-depleted cells, lactate was produced at a rate of 0.81\,\mu\text{mol}\text{ml}^{-1}\text{RBC}\text{h}^{-1}. Lactate accumulated in the presence of all substrates tested (maximum rate, 2.89\,\mu\text{mol}\text{ml}^{-1}\text{RBC}\text{h}^{-1} in the presence of adenosine together with glucose), presumably reflecting the breakdown of glycolytic intermediates (Table 1). The addition of inosine resulted in similar lactate production to that measured in the presence of

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(<a href="%5Cmu%5Ctext%7Bmol%7D%5Ctext%7Bml%7D%5E%7B-1%7D">\text{ATP}</a>)</th>
<th>(<a href="%5Cmu%5Ctext%7Bmol%7D%5Ctext%7Bml%7D%5E%7B-1%7D">\text{Pyruvate}</a>)</th>
<th>(<a href="%5Cmu%5Ctext%7Bmol%7D%5Ctext%7Bml%7D%5E%7B-1%7D">\text{Lactate}</a>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.31±0.09</td>
<td>0.37±0.01*</td>
<td>4.05±0.16</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.17±0.05</td>
<td>0.72±0.01*</td>
<td>8.99±0.54</td>
</tr>
<tr>
<td>Glucose+sodium iodoacetate</td>
<td>0.00±0.00</td>
<td>0.25±0.01</td>
<td>2.09±0.06</td>
</tr>
<tr>
<td>Glucose+ouabain</td>
<td>2.39±0.03</td>
<td>0.57±0.01</td>
<td>2.49±0.07</td>
</tr>
<tr>
<td>Adenosine</td>
<td>4.31±0.10</td>
<td>0.37±0.01*</td>
<td>10.90±2.13</td>
</tr>
<tr>
<td>Adenosine+sodium iodoacetate</td>
<td>0.82±0.03</td>
<td>0.18±0.01</td>
<td>1.09±0.07</td>
</tr>
<tr>
<td>Adenosine+ouabain</td>
<td>3.14±0.13</td>
<td>0.27±0.01</td>
<td>1.48±0.08</td>
</tr>
<tr>
<td>Adenosine+dipyridamole</td>
<td>1.61±0.04</td>
<td>0.14±0.01</td>
<td>2.65±0.01</td>
</tr>
<tr>
<td>Glucose+adenosine</td>
<td>3.48±0.04</td>
<td>1.00±0.01*</td>
<td>12.36±0.26</td>
</tr>
<tr>
<td>Glucose+adenosine+sodium iodoacetate</td>
<td>0.82±0.03</td>
<td>0.18±0.01</td>
<td>1.59±0.06</td>
</tr>
<tr>
<td>Glucose+adenosine+ouabain</td>
<td>3.36±0.13</td>
<td>0.53±0.01</td>
<td>2.61±0.16</td>
</tr>
<tr>
<td>Glucose+adenosine+dipyridamole</td>
<td>2.29±0.10</td>
<td>0.37±0.01</td>
<td>3.10±0.01</td>
</tr>
<tr>
<td>Inosine</td>
<td>1.11±0.05</td>
<td>0.44±0.01*</td>
<td>8.25±0.65</td>
</tr>
<tr>
<td>Inosine+sodium iodoacetate</td>
<td>0.52±0.02</td>
<td>0.17±0.01</td>
<td>2.19±0.10</td>
</tr>
<tr>
<td>Inosine+ouabain</td>
<td>1.35±0.05</td>
<td>0.41±0.01</td>
<td>7.08±0.10</td>
</tr>
<tr>
<td>Inosine+dipyridamole</td>
<td>1.01±0.04</td>
<td>0.18±0.01</td>
<td>3.33±0.01</td>
</tr>
<tr>
<td>Glucose+inosine</td>
<td>1.38±0.12</td>
<td>0.50±0.02*</td>
<td>8.93±0.94</td>
</tr>
<tr>
<td>Glucose+inosine+sodium iodoacetate</td>
<td>0.41±0.03</td>
<td>0.18±0.01</td>
<td>2.28±0.06</td>
</tr>
<tr>
<td>Glucose+inosine+ouabain</td>
<td>1.92±0.11</td>
<td>0.79±0.01</td>
<td>8.38±0.20</td>
</tr>
<tr>
<td>Glucose+inosine+dipyridamole</td>
<td>1.79±0.04</td>
<td>0.26±0.01</td>
<td>2.42±0.05</td>
</tr>
</tbody>
</table>

Erythrocytes were incubated for 4h in the presence of 5\,mmol\text{l}^{-1} of the indicated substrates and 1\,\text{mmol}\text{l}^{-1} sodium iodoacetate, ouabain or dipyridamole where indicated.

The values correspond to the concentrations of the metabolites (\mu\text{mol}\text{ml}^{-1}\text{RBC})\pm\text{s.e.m.} at the end of the incubation time (4h) in 10 different experiments. Each experiment was conducted with 3–5 animals to give one pool of cells.

The initial concentrations (0h) were: ATP, 3.08±0.04\,\mu\text{mol}\text{ml}^{-1}\text{RBC}; pyruvate, 0.14±0.01\,\mu\text{mol}\text{ml}^{-1}\text{RBC}; lactate, 0.81±0.04\,\mu\text{mol}\text{ml}^{-1}\text{medium}.

*Results taken from M. Kaloyianni and K. Moutou (in preparation).
glucose alone (Table 1). Lactate formation was fastest in the presence of adenosine either alone or together with glucose. Lactate formation by cells suspended in glucose with adenosine is less than the sum of lactate produced in glucose-only medium and in adenosine-only medium. These findings suggest that, when encountering glucose and adenosine together, the erythrocyte metabolism responds by using each to a lesser extent than when the substrates are available independently. A similar relationship is observed in the presence of inosine (Table 1). These results indicate that adenosine might have some synergistic effect with glucose on either the rate of glycolysis or the rate of glucose uptake into *Rana ridibunda* erythrocytes.

A further indication of the induction of the glycolytic rate by adenosine is shown in Table 2. The concentrations of all the phosphorylated hexoses (G-6-P, F-6-P and FDP) were increased during incubation in the presence of adenosine together with glucose. Levels of phosphorylated hexoses were unchanged after 4h incubation if the cells were deprived of substrates (Table 2).

Table 1 shows the effect of sodium iodoacetate, an inhibitor of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Lew and Ferreira, 1978), on erythrocytic ATP, pyruvate and lactate levels. The presence of sodium iodoacetate in the medium accelerated the loss of ATP. Additionally, all the metabolites measured showed a marked response to sodium iodoacetate treatment (Table 1). Lactate and pyruvate concentrations were significantly reduced in the presence of either adenosine or inosine when sodium iodoacetate was present. It is therefore probable that upon entry into frog erythrocytes these nucleosides are metabolized through the glycolytic pathway.

Ouabain is known to inhibit active cation transport (Schatzmann, 1953). Table 1 shows the effect of ouabain on glycolytic metabolite levels after 4h of incubation with *Rana ridibunda* erythrocytes. The presence of ouabain strongly influences the formation of ATP and lactate in the presence of adenosine. In contrast, when the cells are suspended in inosine, ouabain causes no change in the concentrations of lactate or ATP.

Dipyridamole inhibits adenosine transport (Ross and Pfleger, 1972), adenosine

---

**Table 2. Effect of substrates on levels of phosphorylated hexoses in *Rana ridibunda* erythrocytes**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Glucose 6-phosphate (μmolml⁻¹)</th>
<th>Fructose 6-phosphate (μmolml⁻¹)</th>
<th>Fructose 1,6-diphosphate (μmolml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>4h</td>
<td>0h</td>
</tr>
<tr>
<td>None</td>
<td>0.05±0.004</td>
<td>0.04±0.005</td>
<td>0.02±0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.05±0.002</td>
<td>0.05±0.004</td>
<td>0.02±0.001</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.04±0.003</td>
<td>0.07±0.005</td>
<td>0.03±0.003</td>
</tr>
<tr>
<td>Glucose + Adenosine</td>
<td>0.05±0.003</td>
<td>0.10±0.015</td>
<td>0.02±0.001</td>
</tr>
</tbody>
</table>

Erythrocytes were incubated for 4h at 25°C in the presence of 5mmoll⁻¹ of the indicated substrates. The values correspond to μmolml⁻¹ RBC±s.e.m. in five different experiments. Each experiment was conducted with 3–5 animals to give one pool of cells.
deaminase and inorganic phosphate movement across the erythrocyte membrane (Bunag et al. 1964; Gerlach et al. 1964). Dipyridamole reduced the increase in erythrocyte lactate concentration in the presence of adenosine, suggesting that this effect was dependent upon the entry of adenosine into the red cells (Table 1). Furthermore, ATP production was inhibited. In contrast, the inhibitor did not modify the effect of inosine on ATP levels (Table 1). The addition of dipyridamole to the incubation medium was more effective at reducing lactate production in the presence of adenosine than in the presence of inosine (Table 1).

Discussion

It is well known that adenosine elicits numerous physiological actions; for example, it increases blood flow (Berne, 1963), inhibits lipolysis (Trost and Stock, 1979), interferes with synaptic transmission (Kuroda et al. 1976) and alters tissue cyclic AMP content (Sattin and Rall, 1970). Furthermore, it has been reported that adenosine stimulates glycolytic flux in isolated perfused rat hearts under normoxia and hypoxia, and is thus important in the minimization of myocardial oxygen demands during periods of oxygen deficiency (Wyatt et al. 1989). Since the pioneering work of Gabrio et al. (1955) on the elixir effect of adenosine on erythrocytes stored under blood-banking conditions, the metabolic functions of adenosine in human erythrocytes have been extensively investigated (Hawkins et al. 1980; Plagemann, 1986).

The nucleated erythrocytes of the amphibian *Rana ridibunda* utilize glucose through the glycolytic pathway as well as the pentose phosphate pathway (Kaloyianni-Dimitriades and Beis, 1984; Kaloyianni and Kalomenopoulou, 1990). In contrast with studies on nucleated erythrocytes of other species (Herman et al. 1962), the Krebs cycle does not seem to be functional in glucose metabolism in *Rana ridibunda* red cells despite the presence of a few mitochondria per cell (Kaloyianni and Kalomenopoulou, 1990). Our results extend the findings on amphibian red cells that suggested a metabolic role for adenosine in human and chicken erythrocytes (Plagemann, 1986; Espinet et al. 1989) and indicate that frog erythrocytes possess an effective enzyme system for adenosine metabolism. The ATP content (Table 1), unlike that of the chicken (Espinet et al. 1989) and some mammalian erythrocytes (Kim et al. 1984), is enhanced by 50% when frog red cells are incubated in the presence of adenosine. Although glucose is an effective metabolic substrate for *Rana ridibunda* erythrocytes, glucose alone could not maintain ATP levels. Additionally, 5mmol l\(^{-1}\) inosine failed to support *de novo* synthesis of ATP. However, the presence of either inosine or glucose alone resulted in lactate accumulation (Table 1). The greatest lactate production measured was in the presence of adenosine, either with or without glucose (Table 1). This suggests that *Rana ridibunda* erythrocytes have the ability to utilize adenosine as a carbon source for lactate formation. Table 1 shows that, when the nucleosides and glucose were present together in the incubation medium, the production of lactate (or ATP) was not the sum of the lactate (or ATP) formed in the presence of either of the two substrates alone. There seems, therefore, to be either some competition between substrates or simply a relationship with the energy demands of the cell. The former could be attributed either to a common mode of transport...
Amphibian erythrocyte metabolism

or to a common enzyme being involved in both pathways. These results corroborate the findings with inosine that the availability of glucose reduces metabolic reliance on a purine nucleoside. The mechanisms by which the two substrates influence the metabolism of each other is poorly understood. It has been found for human erythrocytes that the glucose transporter protein is blocked by the adenosine analogue 8-azidoadenosine (Jarvis et al. 1986; May, 1988).

The significant increase in the levels of glycolytic intermediates during 4h incubation of intact *Rana ridibunda* erythrocytes in the presence of adenosine (Table 2) corroborates the metabolic role of adenosine. The stimulatory effect of purine compounds has already been reported in erythrocytes of other species (Seider and Kim, 1979; Kim, 1983; Kim et al. 1984; Jarvis et al. 1986).

The ability of adenosine to be utilized as a carbon source for lactate formation in *Rana ridibunda* erythrocytes is also confirmed by the observation that sodium iodoacetate suppresses lactate production from adenosine (Table 1). ATP and pyruvate synthesis are also inhibited in the presence of sodium iodoacetate in *Rana ridibunda* erythrocytes. Ouabain inhibited lactate production by erythrocytes suspended in adenosine, but not by cells suspended in inosine (Table 1), suggesting that adenosine uptake into *Rana ridibunda* red cells is related to active cation transport and possibly to Na⁺/K⁺-ATPase.

The conclusion that adenosine is a metabolic substrate and has a sparing effect on glucose uptake and utilization by *Rana ridibunda* erythrocytes is further supported by the results presented in Table 1. An inhibitor of nucleoside transport, dipyridamole, reduced the increase in erythrocyte ATP, pyruvate and lactate concentrations produced by adenosine, suggesting that this effect was dependent upon the entry of adenosine into red cells. The latter suggestion is also supported by the observation that the intracellular ribose content is increased after incubation of *Rana ridibunda* erythrocytes in the presence of adenosine (M. Kaloyianni and K. Moutou, unpublished data). This indicates that *Rana ridibunda* erythrocytes are permeable to adenosine and may possess an enzyme system for the conversion of adenosine to inosine and subsequently to glycolytic intermediates, as has been reported for human erythrocytes (Bontemps et al. 1986). The levels of ATP and lactate indicate that the presence of dipyridamole resulted in a more effective inhibition of adenosine than of inosine transport into *Rana ridibunda* erythrocytes (Table 1). When human erythrocytes were suspended in dipyridamole, a 95% reduction of adenosine influx was observed (Plagemann, 1986).

In human red cells, the metabolic fate of adenosine depends on its concentration in the medium. At low adenosine concentrations, it is converted to ATP, whereas at extracellular concentrations higher than 10 μmol l⁻¹ all adenosine transported into the cells is deaminated (Plagemann et al. 1985). The high concentrations of adenosine used in the present study may have promoted deamination of adenosine rather than phosphorylation upon its entry into frog erythrocytes. Some investigators have suggested that the enzymes adenosine kinase and adenosine deaminase may play a direct role in the transfer of adenosine or its product through the plasma membrane and/or in determining the metabolic fate of the adenosine entering the cell (Agarwal and Parks, 1975; Turnheim et al. 1978).

From the results of the present study it is evident that adenosine stimulated frog
erythrocyte glycolysis. Phosphofructokinase (PFK) is one of the regulatory enzymes of *Rana ridibunda* erythrocyte glycolysis (Kaloyianni-Dimitriades and Beis, 1984). Furthermore, it is known that fructose 2,6-biphosphate (F-2,6-P2) is a potent activator of human as well as of avian erythrocyte PFK (Heylen *et al.* 1982; Carreras *et al.* 1987) and that adenosine increases F-2,6-P2 levels in chicken erythrocytes (Espinet *et al.* 1989). Therefore, the possibility that activation of frog erythrocyte glycolysis by adenosine is mediated through F-2,6-P2 should be considered. F-2,6-P2 was found to be a potent activator of the isolated frog erythrocyte glycolytic enzyme PFK (data not shown). Therefore, an increase in F-2,6-P2 levels, possibly induced by adenosine, could result from an increase in the levels of 6-phosphofructo-2-kinase (PFK-2) substrates and/or effectors or activators of PFK-2 and, subsequently, an induction of glycolysis by activation of PFK.

In conclusion, it is confirmed that adenosine has only a small effect on glucose utilization by frog erythrocytes. Adenosine in the presence of inosine stimulates the glycolytic flux by activating glycolysis, which may be related to cation transport as well as to the possible increase in F-2,6-P2 levels in *Rana ridibunda* erythrocytes. However, the extent to which adenosine functions in this way in red blood cells *in vivo* and whether its metabolism is influenced by oxygen tension and ageing of the cells remain to be investigated.

**References**


Amphibian erythrocyte metabolism


