SUBSTANCE RATES OF DIFFERENT STEPS OF PURINE METABOLISM IN NORMAL AND PRESERVED RED BLOOD CELLS (RBC) STUDIED IN EXPERIMENTS SIMULATING IN VIVO CONDITIONS

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INTRODUCTION

In RBC the energy provided by glycolysis is transferred by ATP to energy requiring processes as ion pumping, chemical synthesis etc by means of ATP. That explains the use of this substance as a marker for cell viability after preservation of erythrocytes and also the addition of precursors - adenine, adenosine, inosine (for the ribose phosphate part) etc - to preservation media. The physiological role of RBC in the transport of purine compounds between the organs of the body has been discussed for years. In order to answer questions hidden within these fields of exploration more firm data describing the substance rates of the specific steps of purine metabolism in RBC are needed.

METHODS

The dialysis system for incubation of RBC, briefly described in earlier publications from these laboratories 1-2 was applied for the investigation of purine metabolism because it allowed continuous and calculable addition and removal of the substances at low - close to physiological - concentrations. The dialysis tubing Cuprophan \(^{\text{R}}\), (thickness 20 \(\mu\)m; width 4 mm) was kindly supplied by Enka AG, P.O.B. 200916, D-5600 Wuppertal 2.

About 900 mm of the tubing was immersed into the RBC suspension. A tubing pump delivered the basic medium with some additives to the affluent end of the dialysis tubing. The volume rate of the pump was measured and kept about 45 ml·h\(^{-1}\). The volume of the suspension was at the start of the experiment about 85 ml. The experiment was carried out in a 100 ml glass beaker equipped with a magnetic stirrer and placed in a water bath thermostated at 37°C.

C. H. M. M. De Bruyn et al. (eds.), Purine Metabolism in Man-IV
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RESULTS AND DISCUSSION

Adenine and Adenosine

Addition of adenine in high concentrations (50 μmol·l⁻¹) to RBC partly deprived of adenylates resulted under physiological conditions in a net synthesis of adenylates at substance rates of 51, 26 and 33 μmol·h⁻¹ (3 experiments). Higher phosphate concentration and simultaneous addition of inosine increased the rate of synthesis two to three times.

Addition of adenosine to comparatively low concentrations resulted in rapid synthesis of adenylates. The proportion of adenosine deaminated to inosine increased at concentrations above the physiological level as shown in Table 1.

Table 1. Metabolism of adenosine added to RBC in the dialysis incubation system. Substance rates calculated per litre RBC

<table>
<thead>
<tr>
<th>Adenosine in susp. μmol·l⁻¹</th>
<th>&lt;0.01</th>
<th>0.04</th>
<th>0.75</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylate, net synthesis, μmol·h⁻¹</td>
<td>50</td>
<td>250</td>
<td>240</td>
<td>340</td>
</tr>
<tr>
<td>Inosine, release, μmol·h⁻¹</td>
<td>0</td>
<td>30</td>
<td>140</td>
<td>250</td>
</tr>
<tr>
<td>Hypoxanthine, release, μmol·h⁻¹</td>
<td>60</td>
<td>430</td>
<td>420</td>
<td>750</td>
</tr>
</tbody>
</table>

Guanosine

Addition of guanosine to RBC suspension resulted in the synthesis of guanylates with an energy charge that was close to the adenylate energy charge. The sum of adenylates + guanylates reached a higher value than the substance concentration of the adenylates alone, probably due to the fact that GTP exerts less product inhibition than ATP at the phosphofructokinase step. Supplementation of blood preservation media with guanosine results in higher 2,3-P₂-glycerate concentration². Our finding that GTP loaded RBC exported hypoxanthine but no guanine indicates that the catabolism passes the guanylate reductase steps and generates oxidized nicotinamide cofactors which are known to stimulate the synthesis of 2,3-P₂-glycerate.

Liquid Preserved RBC

RBC stored at 4°C in CPD-adenine or SAG-mannitol for different periods of time have, after neutralization with isotonic Na-bicarbonate and washing, been studied in the dialysis incubator. Figure 1 illustrates the data obtained for some storage units when the RBC were incubated in a physiological medium for 4 h with a supplementation of adenine in order to prevent purine depletion. Adenylate energy charge is shown to the left and total adenylates to the right with identical symbols for the units in the two diagrams. The letters C and S are used to indicate the