THE FUNCTION OF ADENOSINE DEAMINASE IN THE HUMAN ERYTHROCYTE

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SUMMARY: The metabolism of adenosine and adenine by the intact human erythrocyte has been studied over the range 0.1 to 180 μmol/l. At physiological levels (<1 μmol/l) of adenosine <10% was deaminated by adenosine deaminase, the majority forming adenine nucleotides via adenosine kinase. Above 7 μmol/l deamination became the dominant pathway, increasing until at 180 μmol/l >90% of the adenosine was deaminated. Over the physiological range adenine and adenosine were incorporated into nucleotides at identical rates. The relevance of these findings to human purine metabolism and their implications to adenosine deaminase deficient patients is discussed.

The current interest in purine metabolism stems from reports linking the deficiency of adenosine deaminase (ADA., E.C.3.4.5.4) with severe combined immunodeficiency disease (SCID) in children (1,2). Investigations of erythrocyte ADA deficiency have usually been performed in unphysiological buffers using adenosine concentrations >10 μmol/l. Previously we confirmed that the composition of the incubation medium, in particular the Pi concentration, plays an important role in the overall metabolism of adenine and adenosine by human erythrocytes (3). Only recently have the low levels of these compounds in plasma and cell been measured and the reported plasma levels were 0.64 ± 0.15 μmol/l and 0.31 ± 0.29 μmol/l for adenine and adenosine respectively (4).

Meyskens and Williams (5) first noted the possible importance of the relative Km values for adenosine for the two 'salvage' enzymes, adenosine kinase (E.C. 2.7.1.20) and ADA, both of which occur in the erythrocyte. We previously observed some changes in the relative rates of phosphorylation and deamination of adenosine over the range 10-100 μmol/l. (3). Using high performance liquid chromatography (HPLC), combined with the

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use of high specific activity radio-labelled substrates, we have now studied the metabolism of adenine and adenosine by intact human erythrocytes over a wider substrate range including physiological levels.

METHODS [8-^{14}C] Adenosine (54 Ci/mol), [8-^{14}C] adenine (54 Ci/mol) and [2-^{3}H] adenine (21 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. The methodology has been fully described (3). The incubation buffer had an overall composition of: glucose (5.6mM); NaCl (140mM); $MgSO_4$ (0.98mM): KCl (5.4mM); $CaCl_2$ (2.4mM); Pi (1mM); pH 7.4.

Blood from normal volunteers was immediately centrifuged (3 min at 1000g), the plasma and buffy coat removed and the red cells washed with buffer (2 vol). All incubations were commenced within 20 min of venipuncture. [2-^{3}H] adenine was employed at substrate concentrations below 10μmol/l and [8-^{14}C] adenine for higher concentrations. Red cells (200μl) were incubated at 37^{o} with varying adenine or adenosine concentrations in the buffer (haematocrit 40%). After 5 min the incubations were terminated by addition of 20% w/v trichloracetic acid (100μl) and then neutralized. [^{14}C] labelled bases, nucleosides and nucleotides were separated by high voltage electrophoresis and estimated by scintillation counting. [^{3}H] - labelled nucleotides and bases were separated by HPLC using a slight modification of the rapid (5 min) methodology (6). 1ml fractions were collected and counted in the geling scintillation cocktail (2 ml) of Woods et al (7).

RESULTS The results of incubating human erythrocytes in a buffer of physiological composition with increasing amounts of adenine or adenosine are shown in Fig 1. The only measurable metabolites formed from labelled adenine were ATP, ADP, AMP (in the ratios of 10:1: 0.1) and these pathways were apparently saturated at ca.2 μmol/l adenine. Of the supplied adenine 60% was metabolised at the lowest concentration but only 0.4% at the highest. Adenosine, however, was >98% metabolised at all substrate levels forming AMP + ADP + ATP and inosine. Because inosine was rapidly converted to hypoxanthine, some of which formed IMP, total deamination products have been plotted in Fig 1, but in all cases >98% of this total was hypoxanthine. The amount of adenosine deaminated varied in a sigmoid manner with the substrate level. Below 7μmol/l nucleotide synthesis took precedence, whereas above this value deamination was predominant, accounting for 90% of metabolites at 180 μmol/l. Below 1μmol/l substrate (maximum physiological level), the rate of adenine nucleotide formation was identical whether formed from adenine or adenosine.

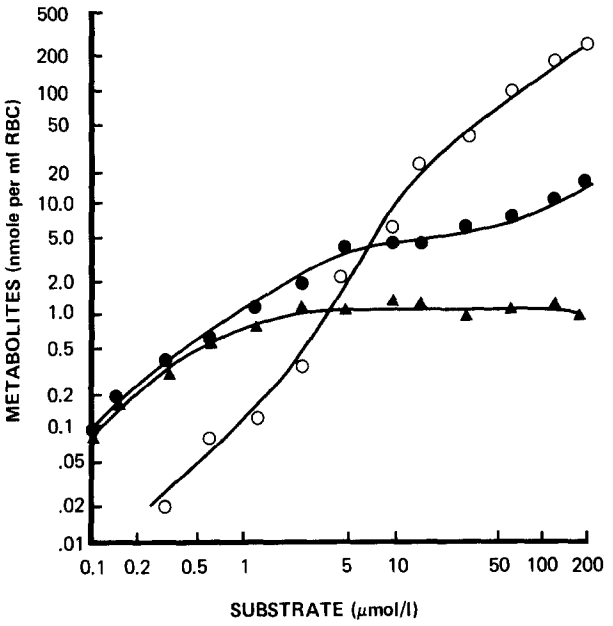

Figure Results of incubations of human erythrocytes with varying labelled adenine or adenosine levels. Conditions as described in Methods. Values are the mean of duplicate observations on erythrocytes from two normal males. (▲ - ▲), ATP + ADP + AMP from adenine; (● - ●), ATP + ADP + AMP from adenosine and (O - O), hypoxanthine + inosine + IMP from adenosine. (N.B. log scales)

DISCUSSION The reason for the association of two rare disorders, one clinical i.e. SCID and the other biochemical i.e. ADA deficiency, first reported by Giblett et al (1), has not yet been elucidated. Various hypotheses have been proposed and the effects of adenosine on the immunological function of various tissues (8,9) has been reported. Mostly the investigations have employed relatively high adenosine concentrations (>10 μmol/l) although physiological levels were reasoned to be lower. Amongst other disadvantages, the use of such high levels in buffers containing 10-50 μmol/l phosphate, leads to the accumulation of abnormally high IMP levels in red cells. The recent combination of column chromatography with a sensitive fluorimetric assay has enabled Mills et al (4) to report the first apparently accurate physiological levels of adenine and adenosine. These levels are at least an order of magnitude below those used by most workers, including ourselves (3). At high adenosine concentrations deamination appeared the major metabolic pathway.

However the present study clearly shows that this is not the case at physiological adenosine concentrations, where deamination accounts for <10% of its metabolism.

The maximum plasma adenosine level (3.6μmol/l) reported by Mills et al (4) occurred in a child with ADA deficiency, and even at this level the normal red cell would deaminate only 20% of the adenosine. Thus for the red cell 'in vitro', ADA plays an apparently insignificant role in adenosine metabolism because at normal plasma levels adenosine is efficiently salvaged for nucleotide synthesis via adenosine Kinase. If this is also the case 'in vivo', the role of ADA in purine metabolism would appear minimal, since the red cell is a major source of the enzyme.

Figure I shows that 1ml of red cells could metabolise 300nmol of adenosine/5 min, equivalent to the formation of 170 mmol/day of inosine or hypoxanthine by the body's total red cell population. This rate exceeds the normal urate excretion by a factor of 20. However, at physiological plasma adenosine concentrations, the red cells would account for <1% of urate production. Only if transient high fluxes of adenosine occur in plasma would deamination by the red cells account for a higher percentage of urinary urate.

Meyskens and Williams (5) postulated that control of adenosine metabolism depends upon the relative Kms for ADA and adenosine Kinase. The present study demonstrates the validity of their analysis for the intact erythrocyte. The initial portions of the curves (see Fig 1) indicate that adenosine Kinase is a low capacity, high affinity enzyme and ADA is a high capacity, low affinity enzyme. It is not clear why an increase occurs in the rate of adenosine Kinase above 20μmol/l substrate. But in this system transport into the cell is a major factor, particularly at high substrate levels when the diffusional component of transport increases. However, in the case of adenine, no corresponding increase was observed. The possibility therefore arises that at high adenosine levels alternative pathways of ATP formation e.g. direct phosphorylation, may become significant.

Although no saturation of the enzymes of adenosine metabolism occurred, AMP: pyrophosphate phosphoribosyltransferase (Ad PRTase, EC 2.4.2.7) was saturated at 5μmol/l adenine after

5 min incubation. It has been shown (10,3) that levels of 'available' P-ribose-PP are a function of both phosphate level and incubation time. In physiological phosphate buffer, P-ribose-PP synthesis corresponded to 14nmol/ml RBC (3) when measured over a 1 hr period. This rate corresponds closely with that determined here which was 1.1nmol/ml RBC/5 min. i.e. 13nmol/ml RBC. The measured levels of P-ribose-PP in human red cells range from 1-5 nmol/ml (11). Therefore, in the present study labelled adenine nucleotide formation apparently did not utilise all the P-ribose-PP pool. Whether the rate of nucleotide synthesis from adenine was limited by (1) transport into the cell, (2) synthesis of P-ribose-PP or (3) the affinity of Ad PRTase for P-ribose-PP and adenine, is not clear. However, the rate of P-ribose-PP synthesis seems more than adequate to cope with physiological adenine concentrations.

Because of the changes in the overall metabolism of adenosine with substrate concentration it would appear necessary to screen for ADA deficiency at adenosine concentrations $>$ 10umol/l. But studies over a wider substrate range must then be performed on ADA-deficient cells to try to further unravel this paradoxical situation which suggests that ADA is of little consequence in normal adenosine metabolism. Unfortunately screening of SCID patients in this hospital has not as yet identified any ADA-deficient subjects.

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