# EVIDENCE AGAINST THE COMPARTMENTATION OF ADENOSINE KINASE AND ADENOSINE DEAMINASE ACTIVITIES IN HUMAN ERYTHROCYTES

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## 1. Introduction

In most tissues, the metabolism of exogenous adenosine is initiated by either adenosine kinase (EC 2.7.1.20) or adenosine deaminase (EC 3.5.4.4) activities. A general finding is that phosphorylation of adenosine is favored at low adenosine concentrations while deamination is favored at higher concentrations [1–4]. Several workers have suggested that this concentration-dependent metabolic fate of adenosine arises from the compartmentation of adenosine kinase activity adjacent to the membrane transport system for adenosine [5–7]. In contrast, results obtained with human red cells incubated in the presence and absence of the nucleoside transport inhibitor *p*-nitrobenzylthioguanosine (2-amino-6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine) led Agarwal and Parks to conclude that adenosine deaminase activity may be closely associated with the nucleoside transporter on the red cell membrane [8]. The simple experiments reported here do not support these hypotheses of compartmentation of either adenosine kinase or adenosine deaminase in human red cells. Rather, our results indicate that the metabolic fate of adenosine in these cells is influenced mainly by the maximal activity and $K_m$ parameters for adenosine kinase and adenosine deaminase.

## 2. Materials and methods

### 2.1. *General*

Details of most of the materials and methods used in this study have been described in [9]. All experiments were performed with washed human erythrocytes (20% hematocrit) in Krebs–Ringer medium containing 25 mM phosphate [9].

### 2.2. *Metabolism of [8-$^{14}$C]adenosine*

Cells were incubated for 80–90 min in the Krebs–Ringer phosphate medium to allow the intracellular phosphate concentration to reach a steady state level of about 7–8 mM [10]. [8-$^{14}$C]Adenosine (Radiochemical Centre, Amersham; 5 Ci/mol) was then added to give the required concentration. In experiments where nucleoside transport inhibitors were used, these were added to a final concentration of 10 μM 10 min prior to the addition of the [8-$^{14}$C]-adenosine.

### 2.3. *Extraction of purines and chromatography*

Samples of erythrocytes were extracted with $HClO_4$, the extracts neutralised and the purines separated by thin-layer chromatography on PEI-cellulose (Macherey-Nagel) as described in [9].

### 2.4. *Quantification of adenosine metabolised via adenosine kinase and adenosine deaminase*

The [8-$^{14}$C]adenosine incorporated into the various purines was quantified by liquid scintillation counting [9]. Because adenylosuccinate synthetase (EC 6.3.4.4) activity is absent from mature human red cells [11,12] and AMP deaminase (EC 3.5.4.6) activity is strongly inhibited in situ under these experimental conditions [9], the metabolism of adenosine in these cells is limited to the pathways shown in fig.1. Therefore, the amount of the [8-$^{14}$C]adenosine which is metabolised via the kinase reaction is given by the sum of counts accumulated in AMP, ADP and ATP, while the amount of adenosine metabolised via

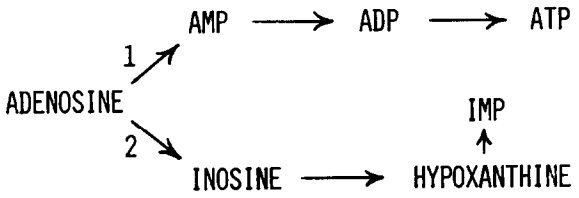


Fig.1. Metabolism of adenosine in human erythrocytes. The enzymes indicated are: 1, adenosine kinase; 2, adenosine deaminase.

the deaminase reaction is given by the sum of counts accumulated in inosine, hypoxanthine and IMP. In the high phosphate medium used in this study, the bulk of the counts were recovered in ATP and IMP.

## 3. Results and discussion

### 3.1. *The metabolism of adenosine in the presence and absence of nucleoside transport inhibitors*

Of a single dose of 50 μM [8-$^{14}$C]adenosine, ~80% was metabolised via the adenosine deaminase reaction while the remaining 20% was incorporated into adenine nucleotides (fig.2A). Under these conditions, all of the adenosine was utilised within 5 min and the end-products of the metabolism (mainly IMP and ATP) were stable for at least 30 min (fig.2A). However, when this experiment was performed in the presence of 10 μM *p*-nitrobenzylthioguanosine, the metabolism of adenosine was considerably slower and the major end-products of adenosine metabolism

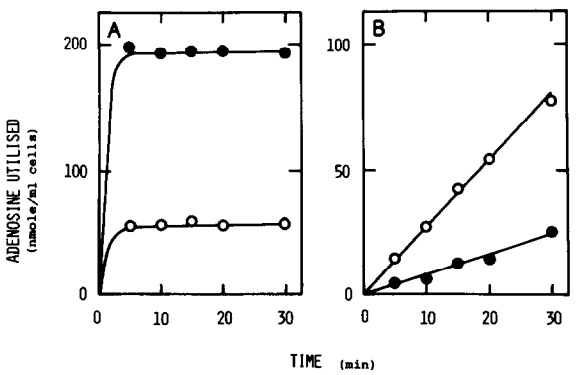


Fig.2. Metabolism of 50 μM adenosine in the absence (A) and presence (B) of 10 μM *p*-nitrobenzylthioguanosine. •, adenosine deaminated; ○, adenosine phosphorylated.

were adenine nucleotides (fig.2B). Essentially identical results were obtained when 10 μM Persantin® (dipyridamole, Boehringer Ingelheim Ltd) was used to inhibit nucleoside transport. Since 10 μM concentrations of these inhibitors prevent uptake of nucleosides via the nucleoside transporter [8,13], the adenosine which entered the cells in this experiment must have done so by passive diffusion through the cell membrane. This very slow passive diffusion would result in a lower intracellular concentration of adenosine. Further, the intracellular metabolism of adenosine is not affected directly by the presence of transport inhibitor as *p*-nitrobenzylthioguanosine does not affect adenosine deaminase activity [8] and we have determined that it does not affect adenosine kinase activity either.

There are two possible interpretations of these results. The adenosine kinase may be located at sites on the membrane which allow passive diffusion of adenosine (conversely, adenosine deaminase may be located adjacent to the nucleoside transporter as suggested by Agarwal and Parks [8]). Alternatively, the factors which determine the metabolic fate of adenosine are independent of the location of the kinase and deaminase but depend instead on the kinetic parameters of these two enzymes. Results which differentiate between these two possibilities are presented below.

### 3.2. *Effect of adenosine concentration on its metabolic fate*

The metabolic fate of 5 μM, 10 μM and 50 μM adenosine is shown in fig.3. To ensure that sufficient counts were incorporated into the various purine metabolites to allow for their accurate estimation, 5 μM 'pulses' of adenosine were added to incubation A at 3-min intervals for a total of 10 'pulses'. Similarly, in incubation B, 5 'pulses' each of 10 μM adenosine were added at 6-min intervals while incubation C received a single 'pulse' of 50 μM adenosine. In this way the same total number of counts was added to each incubation [10]. The results show clearly that phosphorylation of adenosine was favoured at the 5 μM concentration while deamination of adenosine predominated at the 50 μM concentration. Since no transport inhibitor was present in this experiment, the bulk of the adenosine would have entered the cells via the nucleoside transport system in all three incubations. Therefore, the mode of entry of adenosine into the erythrocyte did not play a signifi-

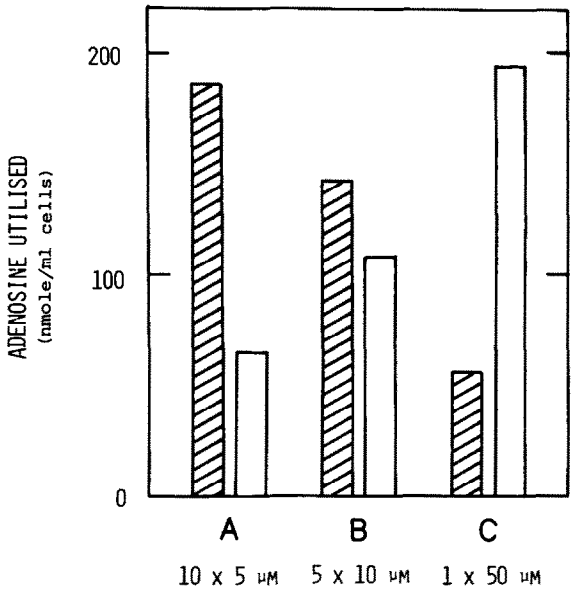


Fig.3. Proportions of adenosine phosphorylated and deaminated at different adenosine concentations. Adenosine was added as follows: (A) 10 'pulses' of 5 μM each at 3-min intervals, (B) 5 'pulses' of 10 μM each at 6-min intervals; (C) 1 'pulse' of 50 μM adenosine. Duplicate samples were taken for extraction and analysis from each incubation 35 min after the addition of the first 'pulse'. The hatched bars represent the amount of adenosine phosphorylated while the open bars represent the amount of adenosine deaminated.

cant role in determining its metabolic fate. Rather, the concentration of adenosine entering the cells appeared to be the more important factor determining the proportions of adenosine phosphorylated and deaminated.

Our results argue against the compartmentation of either adenosine kinase or adenosine deaminase in human erythrocytes. Indeed, all of our data can be interpreted in terms of the maximal activity and $K_m$ parameters for these two enzymes. Adenosine deaminase maximal activity in human erythrocytes is 200–400 nmol/min/ml packed cells [14] and the enzyme exhibits a $K_m$ for adenosine of 30 μM [15]. The adenosine kinase maximal activity is ~40 nmol/min/ml packed cells and the $K_m$ for adenosine is 0.7 μM [16]. From these values, it may be calculated that phosphorylation of adenosine is favoured at the 5 μM concentration while deamination would predominate at the 50 μM concentration.

## Acknowledgement

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