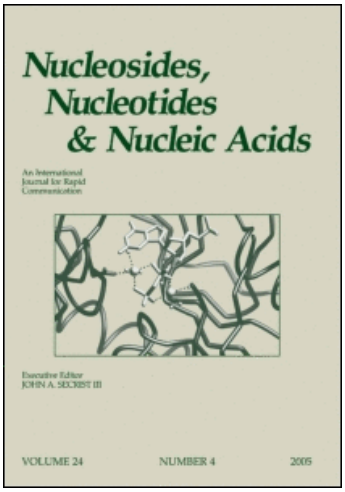

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Metabolism of 4-Pyridone-3-Carboxamide-1-ß-D- Ribonucleoside Triphosphate and Its Nucleoside Precursor in the Erythrocytes

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METABOLISM OF 4-PYRIDONE-3-CARBOXAMIDE-1-ß-D-RIBONUCLEOSIDE TRIPHOSPHATE AND ITS NUCLEOSIDE PRECURSOR IN THE ERYTHROCYTES

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□ *We recently discovered new nucleotides (4-pyridone-3-carboxamide-1-β-D-ribonucleoside phosphates) in human erythrocytes. To establish the precursor compound and pathways of nucleotide derivative formation and breakdown, human erythrocytes were incubated for 3 hours with 0.3 mM 4-pyridone-3-carboxamide-1-β-D-ribonucleoside (4PYR) and erythrocyte concentrations of 4PYR and adenine nucleotides were followed. 4PYR triphosphate increased from 16.1 ± 0.6 μM to 74.9 ± 9.17 and 4PYR monophosphate increased from 5 μM to 254.7 ± 13.9 μM. Conversely, incubation with 0.3 mM 4-pyridone-3-carboxamide (4PY) did not lead to additional 4PYR nucleotide formation. 4PYR nucleotides were catabolized to 4PYR. We conclude that 4PYR nucleotides are formed in erythrocytes by nucleoside kinase-mediated 4PYR phosphorylation and catabolized by 5′ nucleotidase-mediated dephosphorylation.*

Keywords Nicotinamide; NAD; PCNR; 4PYR; ATP; erythrocytes

INTRODUCTION

We recently reported the presence of hitherto unknown nucleotide: 4-pyridone-3-carboxamide-1-β-D-ribonucleoside triphosphate (4PYTP) in the erythrocytes of healthy subjects and its massive accumulation in the erythrocytes of patients with chronic renal failure.[1] This was accompanied by changes in plasma concentration of the nucleoside precursor of 4PYTP: 4-pyridone-3-carboxamide-1-β-D-ribonucleoside (4PYR). Present report evaluates in more details the metabolism of this nucleotide and its

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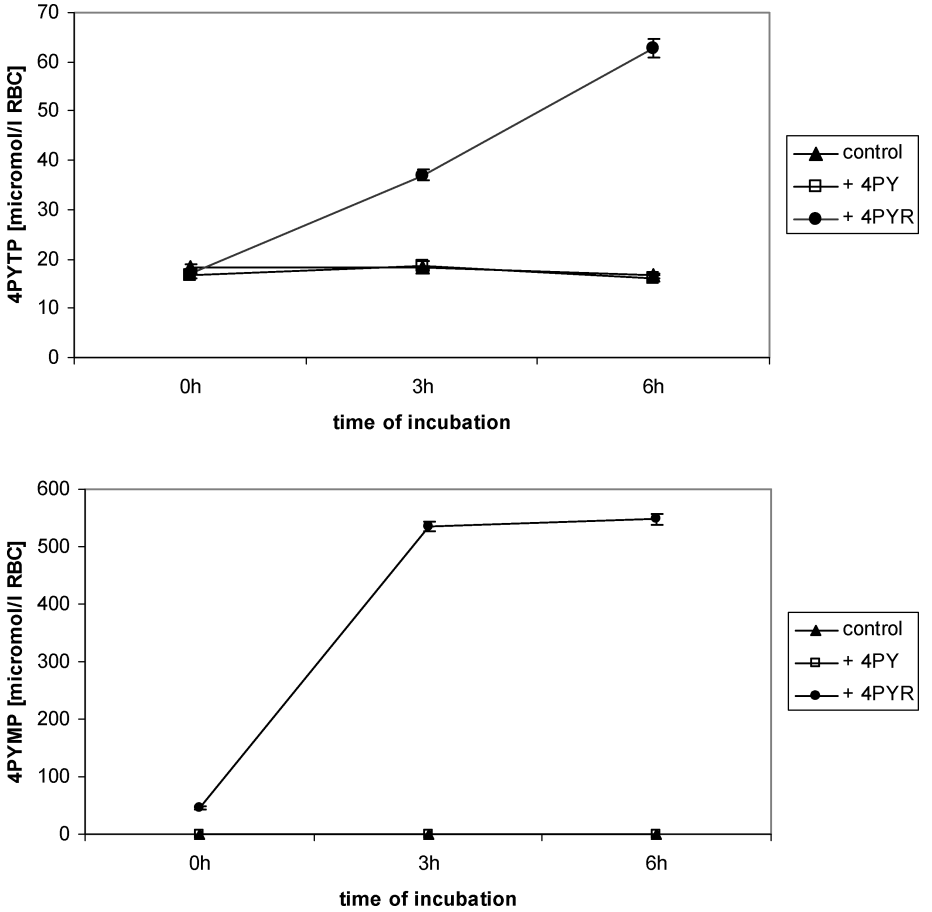

FIGURE 1 Concentration of 4PYTP (top panel) and 4PYMP (bottom panel) in human erythrocytes during incubation with 4PYR or 4PY, indicating progressive accumulation of 4PYTP and 4PYMP with 4PYR but not with 4PY. Values are mean ±SEM, n = 3–6.

precursor in the erythrocytes. This study aimed to establish rates of 4PYR incorporation and factors that affects synthesis of 4PYR nucleotides.

MATERIALS AND METHODS

Chemicals used for this study were obtained from Sigma (Poznan, Poland) with exception of 4PY and 4PYR that were chemically synthesized. Erythrocytes were obtained from healthy volunteers and were washed twice and finally suspended at 20% hematocrit in HEPES buffered Krebs solution. Incubation was carried out at 37°C and was terminated by addition of 0.4 M perchloric acid. Analysis was performed using high performance liquid chromatography as we have described previously.[1]

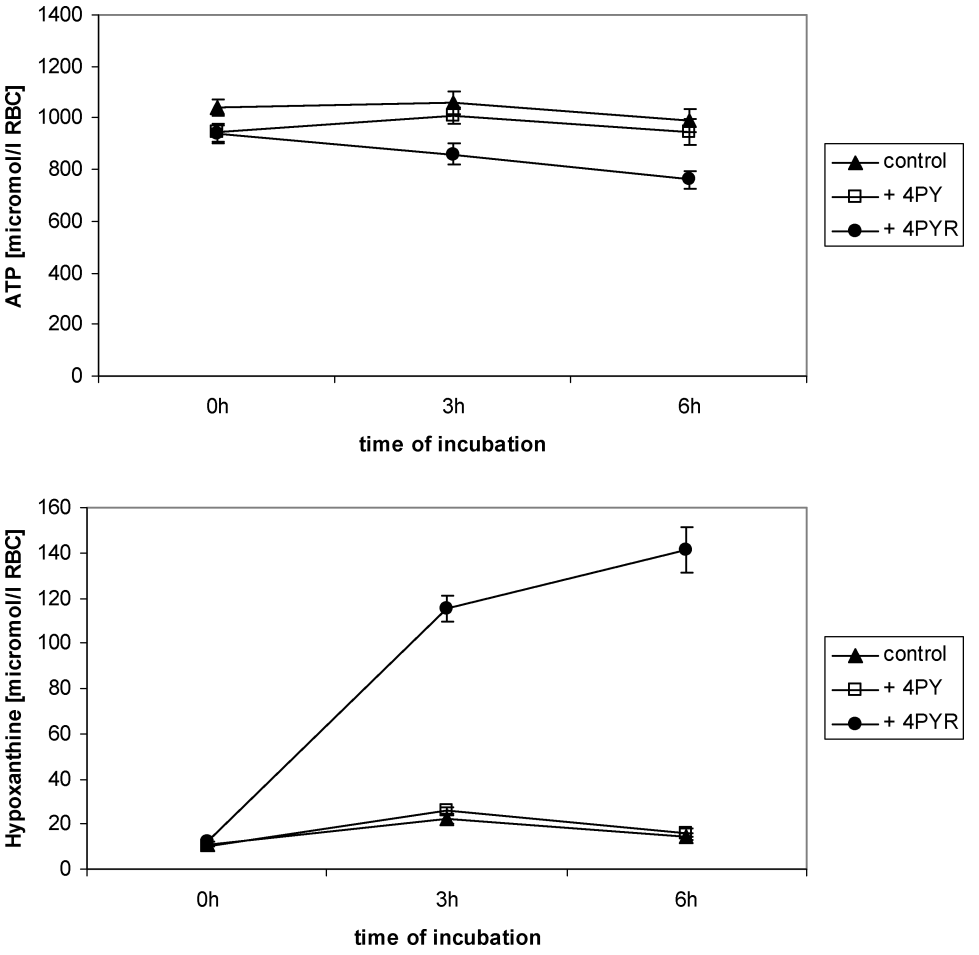

FIGURE 2 Concentration of ATP (TOP PANEL) and hypoxanthine (BOTTOM PANEL) in human erythrocytes during incubation with 4PYR or 4PY indicating decrease in ATP concentration and accumulation of hypoxanthine during incubation with 4PYR. Values are mean ±SEM, n = 3–6.

RESULTS AND DISCUSSION

Figure 1 demonstrates a progressive accumulation of 4PYR nucleotides during incubation of human erythrocytes with 4PYR. No accumulation of 4PYR nucleotides was observed with 4PY. This indicates that 4PYR is a precursor of 4PYTP synthesis in erythrocytes. Accumulation of 4PYMP far exceeded accumulation of 4PYTP (Figure 1). This situation is unusual in nucleotide metabolism as typically nucleoside monophospho- and diphospho- kinases are in excess of nucleoside kinase activity that results in rapid equilibrium of any newly formed monophosphates with ATP/ADP/AMP ratio that in erythrocytes is about 100/10/1. Such a metabolic pattern suggests that this process is aimed to phosphorylate 4PYR and not to form the triphosphate

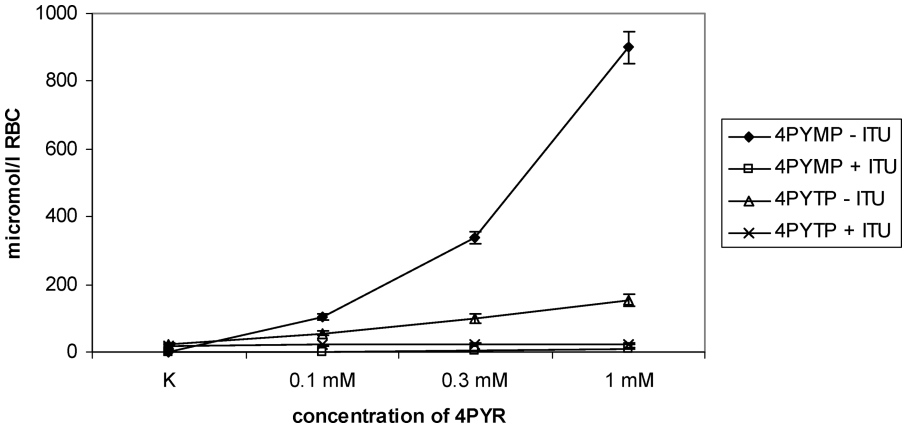

FIGURE 3 Concentrations of 4PYMP and 4PYTP in human erythrocytes incubated for 3 hours with 4PYR at different concentrations with or without 5′iodotubercidin (ITU)—an inhibitor of adenosine kinase—indicating increase in hypoxanthine concentration during incubation with 4PYR. Values are mean ± SEM, n = 3–6.

derivative. Phosphorylation of 4PYR in erythrocytes may prevent its incorporation in other cell types, such as endothelium where 4PYR nucleotides could interfere with nucleic acid metabolism or other cellular processes that require nucleotides.

Figure 2 presents concentrations of ATP and hypoxanthine in erythrocytes exposed to 4PYR. These data indicate that metabolism of 4PYR was associated with depletion of ATP and stimulation of adenine nucleotide degradation in the erythrocytes. This effect was previously demonstrated for several other nucleosides[2] and clearly highlights a potential toxicity mechanism of 4PYR. Such high production of hypoxanthine indicates that 4PYMP could be an activator of AMP deaminase in the erythrocytes, as it cannot be explained by relatively mild increase in AMP concentration (not shown).

Figure 3 shows the formation of 4PYR nucleotides in erythrocytes at different concentrations of 4PYR. Nucleotide formation from 4PYR was completely blocked by 5′-iodotubercidin, indicating that this process was dependent on adenosine kinase activity. However, adenosine kinase seems to have low affinity for 4PYR because even 1 mM 4PYR was not saturating for this process. Thus, the Km for 4PYR seems to be vastly different than the Km for adenosine, which is in the sub-micromolar range.[3]

Figure 4 presents results of experiment that shows erythrocytes are capable of catabolizing 4PYR nucleotides. This experiment indicates that catabolic and anabolic routes of 4PYR nucleotide metabolism have similar rates. 4PYMP that accumulated in erythrocytes during the 3-hour incubation with 4PYR was removed in the subsequent 3 hours of incubation without this precursor. However, 4PYTP was retained, possibly due to a much slower rate of phosphate exchange reactions within the 4PYR nucleotide pool. It is not

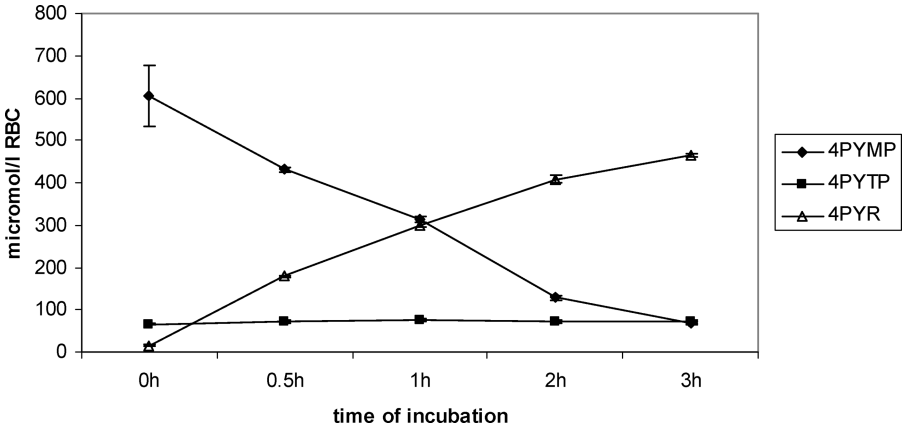

FIGURE 4 Concentrations of 4PYMP, 4PYTP, and 4PYR in human erythrocytes preincubated for 3 hours with 4PYR, washed and incubated for a further 3 hours. Formation of 4PYR but not 4PY indicates that 5′nucleotidase is involved in breakdown of 4PYR nucleotides to form 4PYR. Values are mean ±SEM, n = 3–6.

possible at present to identify which cytosolic 5′-nuclotidase was involved in the production of 4PYR from 4PYMP.

This unusual pattern of 4PYR nucleotides metabolism (that is, closer to nonbiological nucleosides than endogenous compounds) suggests that 4PYR nucleotides are metabolic by-products rather than intermediates serving specific functions. Since dephosphorylation pathway for 4PYMP is of equal capacity to 4PYR phosphorylation, a futile cycling is likely to operate even under normal conditions. It is difficult to propose any specific role of 4PYR nucleotides at present. More information is necessary on the origin of 4PYR in the human body and effect of 4PYR nucleotides on specific enzymes or cellular processes.

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