Volume 254, number 1,2, 5-7 FEB 07511 August 1989

Adenosine formation by isolated rat kidney mitochondria

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Received 22 June 1989

Isolated rat kidney mitochondria are able to generate extraordinary amounts of adenosine. About one-third of the adenosine formed only results from the degradation of adenine nucleotides. Pyridine nucleotides may contribute to adenosine formation. Nevertheless, there must be an additional, as yet unidentified, acid-insoluble compound in mitochondria which is able to form a significant portion of adenosine.

Adenosine; Adenine nucleotide; Mitochondria; (Rat kidney)

1. INTRODUCTION

Several studies have shown that ischemia causes mitochondrial dysfunction [1,2]. At ischemia, the adenine nucleotide contents of heart [3,4] and liver mitochondria [5] decrease. The mechanism of this decrease is not at all understood. Phosphate-induced efflux [6] as well as intramitochondrial degradation of nucleotides has been suggested [5,7]. In previous studies we could demonstrate that isolated mitochondria of rat kidney generate extraordinary amounts of hypoxanthine and inosine [8].

The present study was designed to identify the source of these catabolites. The results provide evidence that adenosine is an obligatory metabolite of adenine nucleotide degradation and that kidney mitochondria are able to form extraordinary amounts of adenosine, additionally.

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2. MATERIALS AND METHODS

Mitochondria were isolated as in [2]. The preparation medium contained 210 mM mannitol and 70 mM sucrose buffered at pH 7.4 with traces of Tris. The incubation experiments with mitochondria were performed in this medium at 37°C for the time periods indicated using about 15 mg protein per ml, 22 μM carboxyatractyloside and 4 μM oligomycin. Where indicated, coformycin was used at 1.3 μM.

Mitochondria were extracted with 0.1 vol. ice-cold perchloric acid (0.33 M). The acid-soluble fraction was neutralized with 0.15 vol. of a solution containing 2 M K_2CO_3 and 0.5 M triethanolamine.

Nucleotides, nucleosides and bases were determined by using a Hewlett Packard (Vienna) 1090 M HPLC system consisting of a ternary solvent delivery system, diode array detector, autosampler and LC workstation. A guard column (2.1 × 20 mm; 10 μm ODS material: Academy of Science, Berlin, GDR) and a microbore analytical column (2.1 × 100 mm; 5 μm ODS Hypersil: Shandon), both manufactured by Hewlett Packard, were used. Chromatography conditions: equilibration period, 10 min using 7% solvent B; linear gradient from 7 to 100% solvent B, taking 30 min; flow rate, 0.1 ml/min. Solvent A: 10 mM $NH_4H_2PO_4$, 0.5 mM tetrabutylammonium bromide (pH 5); solvent B: 10 mM $NH_4H_2PO_4$, 2 mM tetrabutylammonium bromide (pH 6.5), 25% (v/v) acetonitrile.

Protein determinations were made by a Biuret method with human serum albumin as standard [2]. Coformycin, oligomycin and carboxyatractyloside were from Calbiochem AG (Giessen), Serva (Heidelberg) and Boehringer (Mannheim), respectively.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Balance of adenine nucleotides and of catabolites in kidney mitochondria

	Time (min)	Nucleotides (nmol/mg)	Catabolites (nmol/mg)
I	0	5.37 ± 0.07	2.7 ± 0.7
II	60	0.66 ± 0.08	19.7 ± 0.7
III	II-I	4.69 ± 0.05	16.6 ± 1.17

Nucleotides: sum of AMP, ADP and ATP; catabolites: sum of hypoxanthine, inosine and adenosine; incubation time, 60 min; $n = 3$; SD; further details, see section 2

3. RESULTS

Table 1 shows the balance of the extent of adenine nucleotide degradation and of the formation of hypoxanthine, inosine and adenosine. The sum of AMP, ADP and ATP in mitochondria decreased by 4.7 nmol/mg protein when mitochondria were incubated for 60 min. The sum of hypoxanthine, inosine and adenosine increased during this incubation period by 16.6 nmol/mg protein. Consequently, adenine nucleotides were only responsible for 28% of the catabolites formed.

Fig.1 demonstrates two typical HPLC chromatograms. In the absence of coformycin, the main catabolites were hypoxanthine and inosine. However, adenosine was the main catabolite in the presence of coformycin.

4. DISCUSSION

Our results indicate that isolated mitochondria of rat kidney are able to form extraordinary amounts of nucleotide catabolites. The experiments with coformycin, a tight-binding inhibitor of adenosine deaminase [9], show that adenosine is an obligatory metabolite and that, as expected, guanine nucleotides do not contribute markedly to the amount of catabolites formed. The main pathway of adenosine formation may proceed via AMP and 5′-nucleotidase.

The extraordinary amount of adenosine formed could not be explained on the basis of the extent of adenine nucleotide breakdown. The nature of the additional compound(s), containing adenosine as a constituent, remains to be established. The pyridine nucleotides may be partly responsible for the formation of adenosine. In rat liver mitochondria, the content of NAD(P) and NAD(P)H amounts to about 4.5–6.5 nmol/mg protein [10,11]. The degradation of NAD(P) includes the formation of ADP-ribose and AMP, respectively [10–12]. In liver mitochondria, mono(ADP-ribosylation) of proteins by NAD has been described. This potential source of AMP amounts to about 0.2 nmol/mg protein only [12].

Our HPLC analysis yielded no evidence for the existence of any low molecular mass, acid-soluble compound which could be a precursor of

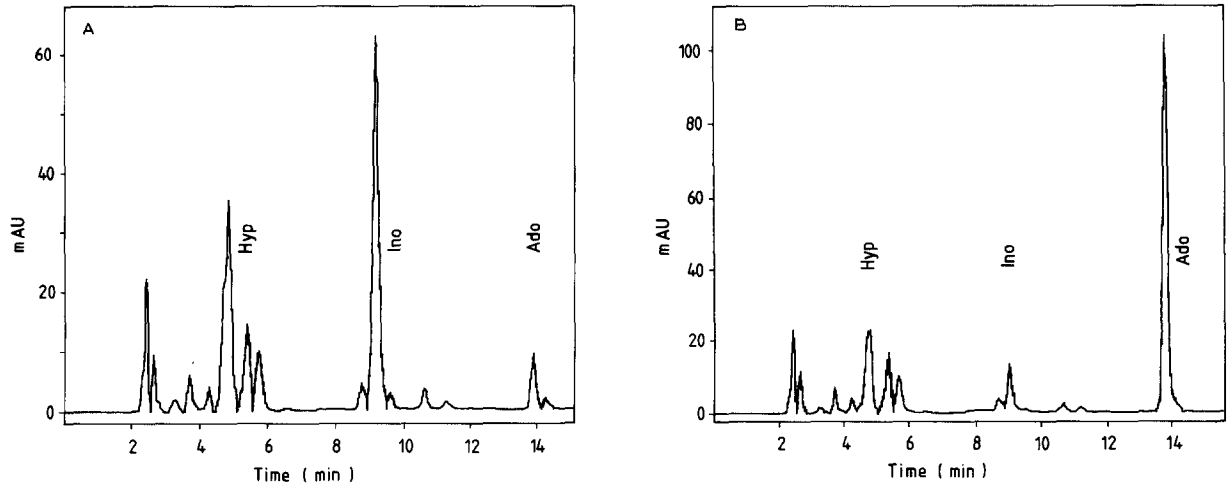

Fig.1. HPLC chromatograms of extracts from incubated kidney mitochondria. (A) Control, (B) with coformycin; incubation time, 15 min; Hyp, Ino and Ado denote hypoxanthine, inosine and adenosine, respectively; further details, see section 2.

adenosine. Recently, in rat heart mitochondria a labelled acid-insoluble compound, presumably either RNA or polyA, was detected after perfusion of Langendorff rat heart with [^{14}C]adenosine [13]. A cyclic process consisting of polyA synthesis by ATP polymerase and polyA degradation by polynucleotide phosphorylase was supposed [14]. By analogy, we assume the existence of a high molecular mass compound, which is responsible for the unusual amount of adenosine formed.

In summary, kidney mitochondria are able to form extraordinary amounts of adenosine. Adenine and pyridine nucleotides may serve as sources of adenosine. A significant proportion of the adenosine formed results from the degradation of a hitherto unidentified, acid-insoluble compound.

Acknowledgements: The work was supported by the Medical Research Project on Chronic Renal Insufficiency of the Ministry of Health of the GDR. We thank Erika Nickel for excellent technical assistance.

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