Biochemical Pharmacology 137 (2017) 107–112

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Catalase increases ethanol oxidation through the purine catabolism in rat liver

Daniel Villalobos-García [a,b], Rolando Hernández-Muñoz [a,*]

[a] Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Ciudad de México 04510, Mexico
[b] Programa de Posgrado en Ciencias Químicas, UNAM, Mexico

ARTICLE INFO

Article history:
Received 26 March 2017
Accepted 15 May 2017
Available online 18 May 2017

Keywords:
Adenosine
Liver slices
Uric acid
Allopurinol
Metabolic conditions

ABSTRACT

Hepatic ethanol oxidation increases according to its concentration and is raised to near-saturation levels of alcohol dehydrogenase (ADH); therefore, re-oxidation of NADH becomes rate limiting in ethanol metabolism by the liver. Adenosine is able to increase liver ethanol oxidation in both *in vivo* and *in vitro* conditions; the enhancement being related with the capacity of the nucleoside to accelerate the transport of cytoplasmic reducing equivalents to mitochondria, by modifying the subcellular distribution of the malate-aspartate shuttle components. In the present study, we explored the putative effects of adenosine and other purines on liver ethanol oxidation mediated by non-ADH pathways. Using the model of high precision-cut rat liver slices, a pronounced increase of ethanol oxidation was found in liver slices incubated with various intermediates of the purine degradation pathway, from adenosine to uric acid (175–230%, over controls). Of these, urate had the strongest (230%), whereas xanthine had the less pronounced effect (178% over controls). The enhancement was not abolished by 4-methylpyrazole, indicating that the effect was independent of alcohol dehydrogenase. Conversely, aminotriazole, a catalase inhibitor, completely abolished the effect, pointing out that this enhanced ethanol oxidation is mediated by catalase activity. It is concluded that the H_2O_2 needed for catalase activity is derived from the oxidation of (hypo)xanthine by xanthine oxidase and the oxidation of urate by uricase. The present and previous data led us to propose that, depending on the metabolic conditions, adenosine might be able to stimulate the metabolism of ethanol through different pathways.

1. Introduction

It has been known for a long time that ethanol oxidation in isolated rat hepatocytes increases according to its concentration, and is raised even to near-saturation levels of alcohol dehydrogenase (ADH) [1]. After inhibition of ADH by pyrazole, approximately 30% of ethanol-oxidizing activity remains associated with non-ADH pathways that also show a dependency on ethanol concentration [1]. Despite participation of non-ADH pathways in liver ethanol oxidation, it is still accepted that NADH re-oxidation in the mitochondrial respiratory chain is the rate-determining factor for ethanol oxidation, as well as for the transport of reducing equivalents into the mitochondria [2,3]. Both ethanol and acetate oxidation increase purine nucleotide degradation [4]. In patients with gout, ethanol increases serum urate levels, whole blood lactate, and the urinary oxypurines value; these results indicate that ethanol increases urate synthesis by enhancing the turnover of adenine nucleotides [5].

Adenosine, precursor of adenine nucleotides, can be considered also a chemical messenger and its action could take place at the level of the same cell (autocrine), the same tissue (paracrine), or in separate organs (endocrine). Moreover, the multi-physiological actions of adenosine could be mediated by several routes, such as by extracellular and intracellular receptors, through its metabolism modulating the methylation pathway and possibly inducing physiological lipoperoxidation (peroxides formation), or by participating in cell energetic homeostasis [6]. This purine nucleoside (200 mg/kg) stimulates the metabolism of ethanol in the whole rat (≈1 mM, assuming a water content of 70%) and in its isolated hepatocytes [7,8]. Here, it was observed that adenosine maintained the normal cytoplasmic redox state of the cell (i.e., the NAD/NADH ratio) in the presence of ethanol, which would require the stimulation of the malate-aspartate shuttle. Therefore, it was concluded that adenosine accelerates the transport of cytoplasmic reducing equivalents to the mitochondria by modifying the subcellular

* Corresponding author at: Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Apdo. Postal 70-243, México 04510, CdMx, Mexico.
E-mail address: rhernand@correo.ifc.unam.mx (R. Hernández-Muñoz).

http://dx.doi.org/10.1016/j.bcp.2017.05.011
0006-2952/

distribution of the malate-aspartate shuttle components [8]. The exact mechanism of action by which adenosine stimulated the malate-aspartate shuttle was, however, not determined, and other non-ADH ethanol oxidizing pathways were not explored. In this regard, it is also accepted that fatty acids generate H_2O_2 via peroxisomal β-oxidation and increase ethanol metabolism markedly in a system that involves catalase-H_2O_2; H_2O_2 generation is sufficient to account for rates of fatty acid-stimulated ethanol metabolism via catalase-H_2O [9].

Therefore, the present study was aimed at exploring the effect of various metabolites of the purine degradation pathway to determine the exact mechanism by which adenosine stimulates the oxidation of ethanol.

2. Material and methods

2.2. Substrates and inhibitors

Adenosine, inosine, hypoxanthine (sodium salt), xanthine (sodium salt), and uric acid (potassium salt) were used as substrates. The inhibitors allopurinol, oxonate (potassium salt), and 4-methylpyrazole were used at a concentration of 1 mM, while aminotriazole was used at 5 mM. All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Animals and incubation technique

Male Wistar rats (250–270 g) fed *ad libitum* were used for all experiments (n = 30). Rats were anesthetized with sodium pentobarbital and the heart was perfused with cold Krebs buffer to remove the blood from the liver. We used the precision-cut rat liver slice model [10], where the organ was removed and sliced with a chopper (0.35 mm thickness). About 400 mg of liver slices was placed in a 25-mL Erlenmeyer flask containing 5 mL of cold Krebs buffer (pH 7.4), as well as 1 mmol/L of any substrate, to which ethanol was added to a final concentration of 10 mmol/L. The buffer was saturated with carbogen (95% O_2/5% CO_2). The flasks were placed in a shaking bath at 37 °C. After a preincubation period of 30 min, one of the substrates was added again to a final concentration of 1 mmol/L and the slices were incubated for 3 h more. Samples of 160 μL were taken from each flask every hour. Perchloric acid at 16% was used to eliminate proteins from the sample, which was later neutralized and used for enzymatic determination of ethanol, xanthine, and urate. All procedures were performed according to the *Federal Regulations for Animals Care and Experimentation* (Ministry of Agriculture, SAGARPA).

2.4. Measurement of ethanol, xanthine, and urate

Ethanol was measured enzymatically with a spectrophotometer. Briefly, 20 μL of NAD (100 mmol/L), 100 μL of thiosemicarbazide (100 mmol/L) and 20 μL of yeast alcohol dehydrogenase (ADH; 988 units/mL) were added to 860 μL of glycine buffer (final volume of 1 mL; pH 9.0). After 10 min incubation, absorbance was read at 340 nm [11]. Xanthine was measured by the appearance of urate at 292 nm using xanthine oxidase. The measurement was corrected considering the partial absorbance overlapping xanthine and uric acid at 292 nm [12]. Urate was measured with the commercial kit URIC ACID-LQ from Spinreact. Lactate was determined by means of an enzymatic assay, as described [13]. The methods used for determining these metabolites had less than 5% of coefficients of variations, detecting a minimum of 2–5 nmoles ml^{-1} and correlation coefficients (r^2) above 0.97.

2.5. Statistics

Statistical significance of the differences was assessed by two-way ANOVA and by a post hoc Newman-Keuls analysis, considering $p < 0.01$ as significant.

3. Results

3.1. Effect of adenosine and its purine by-products on ethanol oxidation by liver slices

Adenosine caused an immediate increase in ethanol metabolism. The peak increase in mean ethanol metabolism rate was 93%, but there was considerable variation between experiments in both the degree and duration of response. Individual experiments' results varied between 50% and 150% increase in rate. This increase lasted between 1 and 2 h (Fig. 1, upper panel). The effects on ethanol metabolism by each activator (purines) are shown in Fig. 1 (lower panel). To determine whether this enhancement in liver ethanol oxidation was exclusive of adenosine, we tested inosine, its deamination metabolite, at the same concentration. Inosine had a similar effect to that of adenosine, both in magnitude and duration (Fig. 1). Since hypoxanthine is the immediate degradation product of inosine, it was tested next at the same concentration as adenosine. It was found that hypoxanthine also had the same effect as adenosine (Fig. 1, bottom). These results could indicate that the lack of ribose attached to hypoxanthine, which is ribose-1-phosphate produced by the purine nucleoside phosphorylase, has no role at all in the effect. However, due to the nucleotide salvage pathway, it was not possible to discard the possibility of an effect mediated by purinergic receptors.

As the hypoxanthine oxidation product, xanthine was also used at the same concentration, causing also a similar effect than that of both hypoxanthine and adenosine (Fig. 1, lower panel). Since hypoxanthine and xanthine had the same effect as adenosine, it became clear that adenosine was not causing the effect directly,

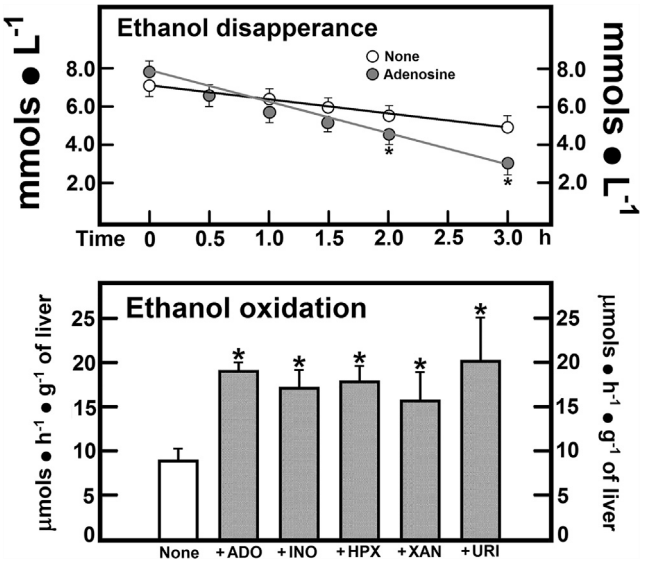

Fig. 1. Effect of adenosine and its purine by-products on ethanol oxidation by liver slices. The upper panel shows typical graphical records for ethanol disappearance, monitored by NAD^+ reduction. In the lower panel, calculated ethanol oxidation by liver slices (preparations from 5 individual rats per experimental group) after the addition of 1 mmol/L of adenosine (ADO), inosine (INO), hypoxanthine (HPX), xanthine (XAN), or uric acid (URI); None addition: purine's vehicle. Experimental groups are designated by symbols at the upper panel of the Figure. Statistical significance: $^*p < 0.05$ vs. control group (no additions).