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Research Report

# AMP deaminase and adenosine deaminase activities in liver and brain regions in acute ammonia intoxication and subacute toxic hepatitis

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ABSTRACT

Cytosolic enzymes AMP deaminase and adenosine deaminase (ADA) catalyze AMP and adenosine deamination, constitute rate-limiting steps of adenine nucleotide catabolism and play important roles in cellular energy metabolism. In this study, AMP deaminase and ADA activities of rat liver, neocortex, cerebellum, striatum and hippocampus were investigated in acute ammonia intoxication and subacute $CCl_4$-induced hepatitis. Activities of both AMP deaminase and ADA in the liver were elevated by 2.4–4.2-fold ($p<0.0001$) in both models of hepatotoxic injury as compared with controls. In acute hyperammonemia activities of AMP, deaminase and ADA increased by 46–59% ($p<0.02$) in the neocortex and did not change in the striatum. In the hippocampus of hyperammonemic rats, only AMP deaminase activity was increased by 48% ($p=0.0004$), and in the cerebellum only ADA activity was increased significantly (by 26%, $p<0.05$). The adenylate pool size and energy charge were greatly reduced in the neocortex of hyperammonemic rats. Results suggested that two parallel pathways of AMP breakdown, including AMP deaminase and ADA, respectively, are up-regulated under pathological conditions, probably in order to overcome compensatory synthesis of adenylates, to ensure prompt adenylate pool depletion and reduce the adenylate energy charge in liver and selected brain regions.



## 1. Introduction

AMP deaminase (EC 3.5.4.6) plays a critical role in energy metabolism. The enzyme catalyzes AMP deamination to give IMP (Fig. 1). Three AMP deaminase isoforms are present in mammals: isoform 1 predominates in skeletal muscle, isoform 2 predominates in liver and brain and isoform 3 seems to be exclusively erythrocytic enzyme.

Adenosine deaminase (EC 3.5.4.4, ADA) is found in all tissues. It catalyzes deamination of adenosine, a product of AMP dephosphorylation (Fig. 1). ADA deficiency in humans is an autosomal recessive disorder that results in severe combined immunodeficiency disease. ADA-deficient mice generated by targeted gene disruption die perinatally (Blackburn et al., 1996).

The two enzymatic reactions are irreversible (Challa et al., 1999; Houston, 2006; Riksen et al., 2008), constitute rate-limiting

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*Abbreviations:* ADA, adenosine deaminase; AN, adenylate pool size; EC, adenylate energy charge

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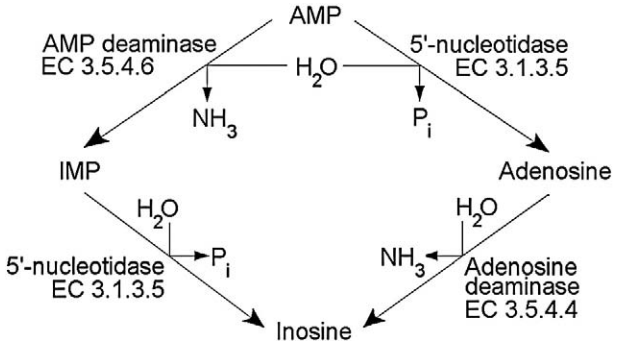


**Fig. 1 – Schematic representation of AMP degradation.**

steps of adenine nucleotide catabolism (Van Den Berghe et al., 1977) and involved in the regulation of the adenylate pool size (AN) and adenylate energy charge (EC) in mammalian cells.

Hepatic ATP is depleted in acute intoxication with ammonium acetate (Kosenko et al., 1994) as well as in $CCl_4$-induced hepatitis (Matkhanov and Kosenko, 1978). Brain ATP, AN and EC are greatly decreased in acute ammonia intoxication, too (Kosenko et al., 1994; Kosenko et al., 2003; Kaminsky and Kosenko, 2009).

In recent study, we observed the dramatic accumulation of xanthine and hypoxanthine in hyperammonemic rat brain, as compared with control values, in parallel with depletion of AN and a drop of EC. We hypothesized that a balance between adenine nucleotide biosynthesis and catabolism is redirected preferentially to catabolic pathway in acute hyperammonemia and suggested that increasing activities of AMP deaminase and ADA could account for adenine nucleotide breakdown and xanthine and hypoxanthine accumulation (Kaminsky and Kosenko, 2009). Such hypothesis was not tested up to now. Moreover, liver and brain AMP deaminase and ADA activities remain largely unexplored in acute hyperammonemia and hepatitis.

Impairment of the cellular energy metabolism seems to contribute similarly to a number of hepatopathies, such as fatty liver (Caraceni et al., 2004), nonalcoholic steatohepatitis (Jaeschke et al., 2002), ammonia-induced (Kosenko et al., 1997) and $CCl_4$-induced hepatotoxicity (Matkhanov and Kosenko, 1978). Therefore, we aimed to determine whether hepatic and brain AMP deaminase and ADA activities change in acute ammonia intoxications and subacute $CCl_4$-induced hepatitis in rats. Additionally, brain adenine nucleotides were measured and the AN and EC were calculated.

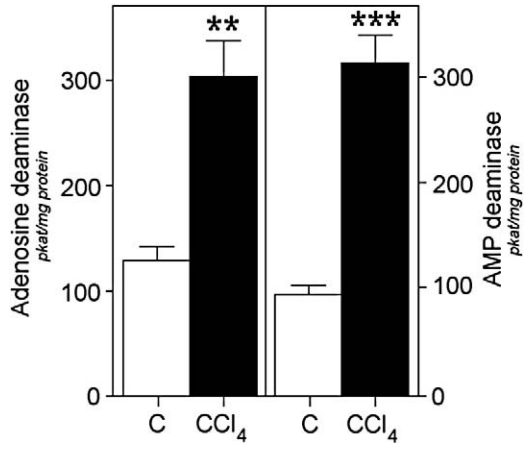


**Fig. 2 – Activities of ADA and AMP deaminase in liver cytosolic fraction from control rats (C) and rats with $CCl_4$-induced hepatitis. Rats were injected with either 0.25 ml of sunflower oil (C) or $CCl_4$ at a dose of 0.25 ml of 40% sunflower oil solution per 100 g body mass on days 1, 3 and 5 ($CCl_4$). Four weeks after the last injection, animals were decapitated, the liver cytosolic fraction was prepared and activities of ADA and AMP deaminase were measured and expressed as pkat/mg cytosolic protein. Results are mean ± S.E.M. of 5 rats (**$p<0.002$, ***$p<0.0001$, as compared to control, Student's t-test).**

## 2. Results

Activities of AMP deaminase and ADA in liver cytosolic fraction from rats with $CCl_4$-induced hepatitis are shown in Fig. 2, and those from hyperammonemic rats in Fig. 3.

Subacute hepatitis led to 135% increase in hepatic ADA activity and 228% increase in AMP deaminase activity ($p<0.0001$) as compared with control levels (Fig. 2).

In hyperammonemic rats, hepatic AMP deaminase and ADA activities were 4.2- and 3.2-fold higher ($p<0.0001$), respectively, than in control animals (Fig. 3).

Activities of AMP deaminase and ADA in the cytosolic fraction from brain regions of hyperammonemic rats are shown in Figs. 4–7.

Activities of AMP deaminase and ADA in the neocortex from hyperammonemic rats were 59% ($p<0.01$) and 46% ($p<0.02$) higher, respectively, than in control rats (Fig. 4).

Activity of ADA in the cerebellum increased by 26% ($P<0.05$) in acute hyperammonemia as compared to control levels while AMP deaminase activity was unchanged (Fig. 5).

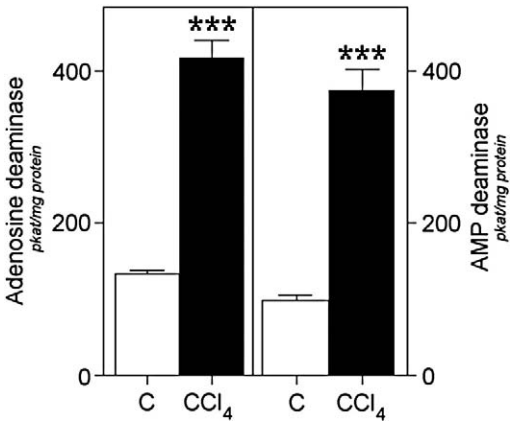


**Fig. 3 – Activities of ADA and AMP deaminase in liver cytosolic fraction from control and hyperammonemic rats. Rats were injected with either saline (C) or 12 mmol/kg of ammonium acetate ($NH_4$). Eleven minutes later, animals were decapitated, the liver cytosolic fraction was prepared and activities of ADA and AMP deaminase were measured and expressed as pkat/mg cytosolic protein. Results are mean ± S.E.M. of 6 rats (***$p<0.0001$, as compared to control, Student's t-test).**