Activation of AMP Deaminase and Adenosine Deaminase in the Liver during Ammonia Poisoning and Hepatitis

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We studied the effect of acute ammonia poisoning and CCl_4-induced subacute hepatitis on activities of AMP deaminase and adenosine deaminase in rat liver. Both models of liver failure were accompanied by an increase in activities of AMP deaminase and adenosine deaminase in the cytoplasmic fraction of the liver (by 2.4-4.2 times compared to the control). A direct correlation was found between activities of AMP deaminase and adenosine deaminase. We believe that two parallel pathways of AMP degradation are activated simultaneously, which leads to rapid depletion of adenylate reserves under pathological conditions.

Key Words: *AMP deaminase; adenosine deaminase; hepatitis; hyperammonemia; liver*

AMP deaminase (EC 3.5.4.6) plays a key role in energy metabolism. This enzyme catalyzes the deamination of AMP to IMP. AMP deaminase is involved in the rate-limiting stage of adenine nucleotide catabolism. Isoforms 1 and 3 of AMP deaminase are mainly present in skeletal muscles and erythrocytes, respectively. Isoform 2 is present in smooth muscles and nonmuscle tissues and regulates the intracellular content of adenylates.

Adenosine deaminase (EC 3.5.4.4) catalyzes the deamination of adenosine, which serves as a dephosphorylated product of AMP degradation). Adenosine deaminase deficiency in humans is an autosomal recessive disorder, which results in severe immunodeficiency. The mice with abnormalities of the enzyme gene die perinatally [1]. Both enzymatic reactions are accompanied by ammonia release, which contributes to the accumulation of this compound.

Hyperammonemia accompanies a variety of liver dysfunctions. This state can be induced by ammonia injection to experimental animals. Ammonia in overdoses serves as a toxin and impairs energy metabolism and transformation of carbohydrates, lipids, and amino acids in the liver [5]. The mechanisms of ammonia hepatotoxicity remain unclear. AMP content decreases in the liver of animals with CCl_4-induced hepatitis and associated hyperammonemia [3]. Acute hyperammonemia is followed by ATP depletion in the brain due to high activity of Na^+/K^+-ATPase and increased consumption of ATP [6]. Abnormal decrease in intracellular ATP can be related to the increased consumption of this substance in metabolic and transport processes. It can be also associated with the catabolism of adenine nucleotides due to a simultaneous action of AMP deaminase and adenosine deaminase. This hypothesis was not tested. Little is known about changes in activities of AMP deaminase and adenosine deaminase in the liver of animal with hyperammonemia and hepatitis.

Here we studied the effect of acute hyperammonemia and subacute experimental hepatitis on activities of AMP deaminase and adenosine deaminase in rat liver.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats aging 2.5-3 months.

Acute ammonia poisoning in rats (n=6) was induced by single intraperitoneal injection of ammonium

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acetate in a lethal dose of 12 mmol/kg. The animals were decapitated 11 min after treatment (or after the first episode of seizure). Control rats (n=6) received the same dose of sodium acetate.

Hepatitis in rats (n=5) was induced by a three-fold subcutaneous injection of 0.25 ml 40% solution of CCl_4 in olive oil per 100 g body weight (days 1, 3, and 5). Control rats (n=5) were treated by 0.25 ml olive oil in the same period. These animals were decapitated 4 weeks after the last injection.

The anterior hepatic lobe was removed within 7 sec after decapitation. The cytoplasmic fraction was obtained by differential centrifugation. AMP deaminase activity in this fraction of the liver was measured spectrophotometrically at 340 nm and 37°C. Enzyme activity was estimated from ammonia release and NADH oxidation in a coupled reaction with glutamate dehydrogenase. The enzymatic reaction was induced by addition of AMP in a dose of 50 mM. Adenosine deaminase activity was measured under similar conditions. However, the reaction was activated by 1 mM adenosine.

Protein concentration was measured by the Lowry method [7] using BSA as a standard.

The results were analyzed statistically with Prizm 4.0 software. The data were presented as means and standard errors. The significance of between-group differences was evaluated by ANOVA (Student's t test).

RESULTS

Activities of AMP deaminase and adenosine deaminase in the liver were elevated by 3.2 and 2.4 times, respectively, during subacute experimental hepatitis (as compared to the control; Fig. 1). Acute ammonia poisoning was accompanied by an increase in activities of AMP deaminase and adenosine deaminase in the liver (by 4.2 and 3.2 times, respectively; Fig. 2).

Activities of AMP deaminase and adenosine deaminase increased in all experimental animals. A direct correlation was found between activities of AMP deaminase and adenosine deaminase (Fig. 3).

Activities of glutamate dehydrogenase (EC 1.4.1.3) and glutaminase (EC 3.5.1.2) in liver mitochondria, activity of glutamine synthetase (EC 6.3.1.2) in the liver cytoplasm, and activities of ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) in liver mitochondria and cytoplasm did not change during acute hyperammonemia and subacute liver poisoning (data not shown).

Similar, but not the same results were obtained on other models of hyperammonemia and liver diseases. AMP deaminase activity was increased in the liver of rats with dietary carcinogen-induced hepatocarcinogenesis and grafted hepatoma (by 1.5-2 and 5.5 times, respectively). Enzyme imbalance was typical of the neoplasm, but not of the regenerating liver [8].

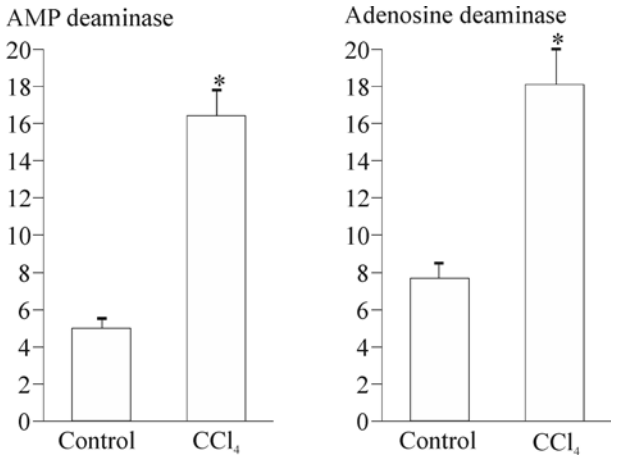

Fig. 1. Activities of AMP deaminase and adenosine deaminase (nmol/mg protein/min) in the cytoplasmic fraction of the liver from rats with experimental hepatitis. Here and in Fig. 2: *p<0.0001 compared to the control.

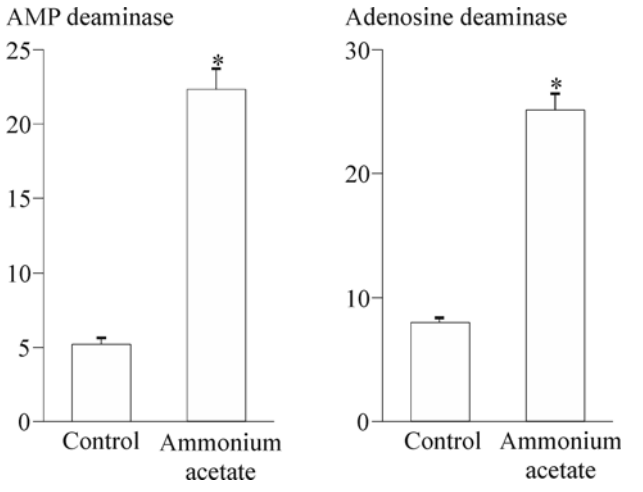

Fig. 2. Activities of AMP deaminase and adenosine deaminase (nmol/mg protein/min) in the cytoplasmic fraction of the liver from rats with ammonia poisoning.

Adenosine deaminase activity increases in the liver of cirrhotic patients [2], heroin-receiving rats [9], and hepatitis B patients [4].

Hyperammonemia is observed in many diseases associated with liver dysfunction (cirrhosis, hepatitis, hepatic encephalopathy, alcoholic intoxication, *etc*.). CCl_4 poisoning serves as the standard model of hepatitis B. Acute ammonia intoxication is a model of hepatic encephalopathy. Our results indicate that activities of AMP deaminase and adenosine deaminase increase manifold in two models of liver injury accompanied by hyperammonemia. The process is specific, because activities of the other five enzymes involved in ammonia formation and neutralization do not change under these conditions. A correlation exists between activities of two key enzymes of purine metabolism. The data suggest that two pathways of

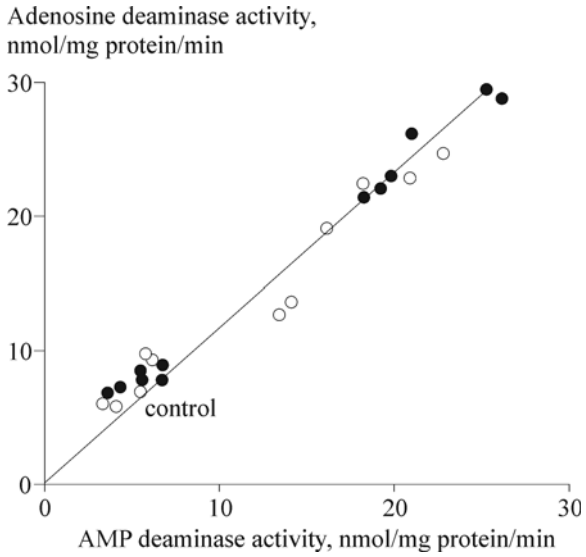

Fig. 3. Correlation between activities of AMP deaminase and adenosine deaminase. Each circle illustrates activities of both enzymes in the cytoplasmic fraction from an individual animal. The regression line is calculated with CurveExpert 1.3 software. The linear correlation coefficient is r=0.976. Light symbols, CCl_4; dark symbols, ammonium acetate.

AMP degradation are activated simultaneously. This effect provides rapid depletion of adenylate reserves under pathological conditions.

Our results and published data show that an increase in activities of two deaminases in the liver is a general process in liver failure.

The molecular mechanisms for abnormalities of AMP deaminase and adenosine deaminase and role of these enzymes in the pathogenesis or adaptation of cells to hyperammonemia require further investigations.

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