Free Radical Oxidation and Catalytic Activity of Aconitate Hydratase in Rat Liver under Normal Conditions and during Toxic Hepatitis

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We observed intensification of free radical oxidation, decrease in activity, and changes in catalytic properties of aconitate hydratase in the liver of rats with toxic hepatitis. The total yield and maximum flash intensity of biochemiluminescence increased by 2.2 and 1.7 times, respectively. Differences were revealed in the regulation of aconitate hydratase activity with Fe^{2+}, Ca^{2+}, H_2O_2, and oxidized and reduced glutathione in the liver of rats with toxic hepatitis and control animals.

Key Words: *free radical oxidation; aconitate hydratase; regulation of activity; rat liver; toxic hepatitis*

Reactive oxygen species (ROS) are produced during normal metabolic reactions in cells. However, their content sharply increases under conditions of insufficient oxygen supply to tissues [8]. Excessive accumulation of ROS and intensification of free radical oxidation (FRO) are accompanied by changes in biological membranes and functional activity of cells, which leads to oxidative stress [1,4,6]. Production of toxic oxygen radicals increases during liver diseases of different etiology [2,9]. The rate of FRO depends on Fe^{2+} concentration. These ions are involved in generation of hydroxyl radicals initiating chain oxidation of biological molecules [1]. Multilevel system of antioxidant defense protects cells from ROS [1,3,4,10]. It is hypothesized that antioxidant potential depends on the concentration of citrate. This compound determines the intensity of hydroxyl radical formation in the Fenton reaction, which involves H_2O_2 and Fe^{2+} as substrates. Citrate acts as a chelator for Fe^{2+} [11]. Aconitate hydratase (EC 4.2.1.3, AH) catalyzing transformation of citrate into isocitrate is of particular interest in this respect. Little is known about functional activity and role of AH in the regulation of ROS content during liver diseases via modulation of intracellular citrate concentration. Here we studied the intensity of FRO and measured catalytic activity of AH in the liver of rats under normal conditions and during experimental toxic hepatitis.

MATERIALS AND METHODS

Experiments were performed on male albino rats (*Rattus rattus L.*) weighing 200-250 g. Each experimental series was conducted on 6-10 animals. Experimental toxic hepatitis was modeled by treatment with the hepatotropic toxin CCl_4 [7]. The liver was removed from narcotized animals after perfusion with physiological saline.

Subcellular fractions were separated by differential centrifugation [6]. The intensity of FRO under normal conditions and during toxic hepatitis was estimated by the method of Fe^{2+}-induced biochemiluminescence [3]. AH activity was measured spectrophotometrically at 240 nm in a medium containing 0.05 M Tris-HCl buffer (pH 8.0) and 4 mM citrate. Purifica-

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tion of AH included fractionation with $(NH_4)_2SO_4$, gel filtration on Sephadex G-25, and ion-exchange chromatography on DEAE cellulose. Enzyme preparations of AH were obtained from the liver of intact rats and animals with experimental toxic hepatitis (specific activity 10.9 and 7.9 U/mg, respectively) after 49- and 68-fold purification. These enzyme preparations were used to compare catalytic activity of AH under normal conditions and during toxic hepatitis. Experiments were performed in 3-4 repetitions. Each sample was assayed twice.

The data had normal distribution and were analyzed by Student's *t* test.

RESULTS

Parameters of biochemiluminescence reflecting the intensity of FRO (total yield and maximum flash intensity) increased by 2.2 and 1.7 times, respectively, in the cytoplasmic fraction of hepatocytes from rats with toxic hepatitis (Table 1). The tangent of the kinetic curve slope ($tg\alpha_2$) characterizing antioxidant potential increased by 33% in animals with toxic hepatitis. AH activity in the cytoplasmic fraction of liver cells from rats with toxic hepatitis decreased by 50% compared to the control.

TABLE 1. Parameters of Biochemiluminescence in the Liver of Control Rats and Animals with Toxic Hepatitis (*M*±*m*)

Index	Normal	Pathology
Total yield, mV×sec	19.28±0.96	41.38±2.06
Flash intensity, mV	1.95±0.10	3.32±0.16
$tg\alpha_2$, mV×sec	3.09±0.15	4.12±0.20

Comparison of enzyme preparations showed that Fe^{2+} activate this enzyme in control rats and animals with toxic hepatitis. In control rats the stimulatory effect of Fe^{2+} in concentrations <1 mM was less pronounced than in animals with toxic hepatitis. By contrast, Fe^{2+} in high concentrations more significantly activated AH from rats with toxic hepatitis (Fig. 1, *a*). Ca^{2+} plays an important role in metabolic changes during oxidative stress and FRO. These ions in concen-

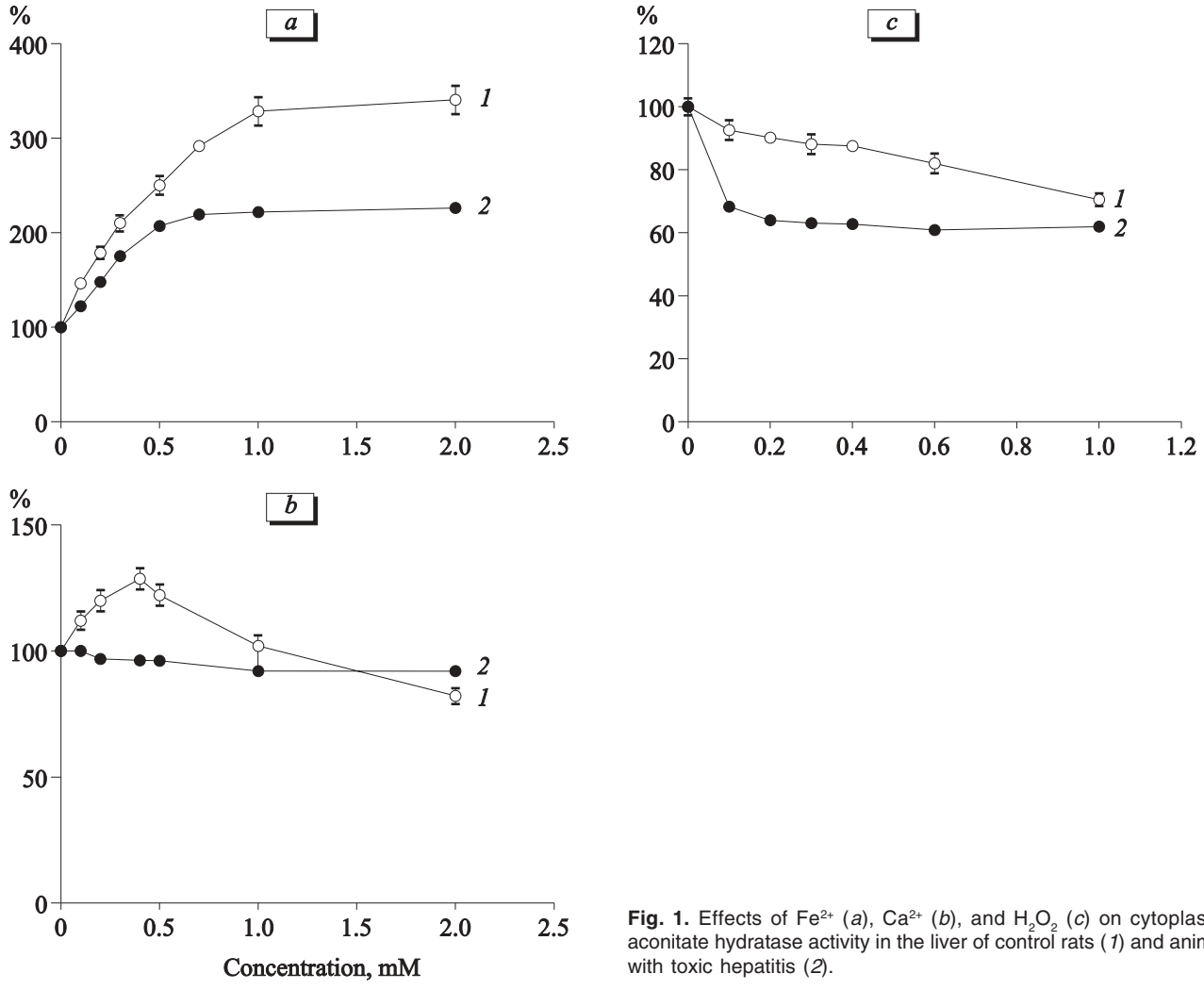

Fig. 1. Effects of Fe^{2+} (*a*), Ca^{2+} (*b*), and H_2O_2 (*c*) on cytoplasmic aconitate hydratase activity in the liver of control rats (*1*) and animals with toxic hepatitis (*2*).

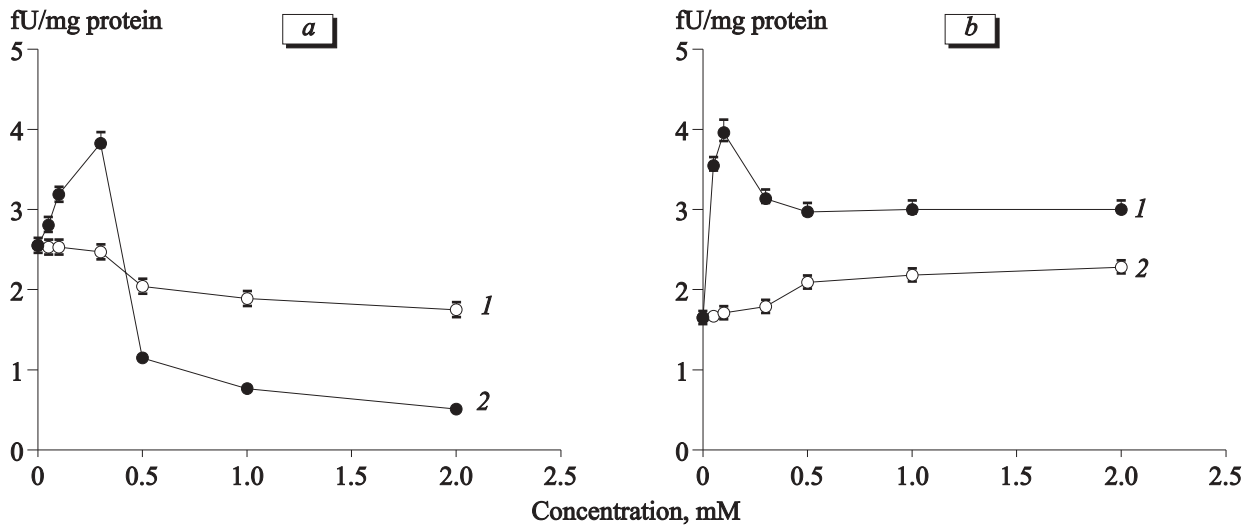

Fig. 2. Effects of components from the glutathione reductase/glutathione peroxidase antioxidant system on aconitate hydratase activity in the liver of control rats (*1*) and animals with toxic hepatitis (*2*): reduced glutathione (*a*) and oxidized glutathione (*b*).

trations <0.3 mmol/liter activated AH from control animals. However, activation of AH was less pronounced under the influence of Ca^{2+} in high concentrations. It should be emphasized that Ca^{2+} slightly inhibited AH from the liver of rats with toxic hepatitis (Fig. 1, *b*). H_2O_2 inhibited AH, which was most pronounced in control animals (Fig. 1, *c*). It was interesting to study the effect of components from the glutathione reductase/glutathione peroxidase antioxidant system on AH activity, because isocitrate, a product of an AH-catalyzed reaction, serves as a substrate for NADP-isocitrate dehydrogenase. The enzyme catalyzes oxidative decarboxylation of isocitrate into 2-hydroxyglutarate. This reaction yields NADPH, which is essential for the glutathione reductase/glutathione peroxidase antioxidant system involved in detoxification of H_2O_2. Oxidized glutathione sharply activated AH from the liver of rats with toxic hepatitis, whereas in control preparations weak activation was observed only at concentrations >0.4 mM. It is important that oxidized glutathione in concentration <0.4 mM was most potent in activating AH from animals with toxic hepatitis (by 1.8-2.2 times). Reduced glutathione in the same concentration range (<0.4 mM) activated AH from the liver of rats with toxic hepatitis. Increasing the concentration of reduced glutathione was accompanied by a sharp decrease in enzyme activity. It should be noted that the inhibitory effect of reduced glutathione in high concentration was more pronounced in animals with toxic hepatitis compared to controls (Fig. 2). Therefore, AH activity depends on the concentration of oxidized and reduced glutathione. It cannot be excluded that pronounced activation of AH with glutathione under pathological conditions led to more intensive production of isocitrate for NADPH-isocitrate dehydrogenase. However, glutathione in high concentrations inhibits AH.

Our findings suggest that FRO intensity markedly increases during toxic hepatitis, which is accompanied by inactivation of AH and changes in the regulation of enzyme activity with H_2O_2, Fe^{2+}, Ca^{2+}, and oxidized and reduced glutathione. Inhibition of AH probably contributes to intracellular accumulation of citrate and inhibition of the Fenton reaction. These specific features of regulation of AH under pathological conditions suggest that enzyme function is coordinated with activity of the glutathione reductase/glutathione peroxidase antioxidant system.

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