Effect of Carbon Tetrachloride on 3′-AMP-Forming Enzyme Activity in Rat Liver

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The effect of carbon tetrachloride (CCl_4) on adenosine 3′-monophosphate (3′-AMP) content and 3′-AMP-forming enzyme activity in rat liver was examined. The levels of 3′-AMP and the total amounts of adenosine 5′-triphosphate, adenosine 5′-diphosphate and adenosine 5′-monophosphate were decreased by treatment with CCl_4. The 3′-AMP-forming enzyme activity in CCl_4-treated rat liver homogenate was *ca.* 4-fold higher than that of control. The enzyme activity in CCl_4-treated mitochondria was *ca.* 50% of control values. These results suggest that the increase in the 3′-AMP-forming enzyme activity in the cytosol may be, at least partly, due to the leakage of the enzyme activity from mitochondria in acute hepatitis induced by CCl_4.

Key words —— 3′-AMP, 3′-AMP-forming enzyme, carbon tetrachloride, rat liver

INTRODUCTION

Adenylate cyclase can be activated or inhibited by a number of extracellular and intracellular signals. Intracellular adenosine 3′-monophosphate (3′-AMP), one of the degradation products of various RNAs, has been pharmacologically classified as a P-site inhibitor of adenylate cyclase.[1] The 3′-AMP-forming enzyme, one of the ribonucleases (RNase), is known to exist not only in homogenates of several organs in rat[2] but also in the mitochondrial fraction of rat liver.[3,4] The biological roles of 3′-AMP and 3′-AMP-forming enzyme have not yet been elucidated.

Carbon tetrachloride (CCl_4) is frequently used as a chemical inducer of hepatic necrosis and fatty liver. Hepatic necrosis is accompanied not only by the inhibition of mitochondrial respiration, protein and DNA synthesis, and adenylate cyclase but also by an increase in intracellular Ca^{2+} and release of intracellular enzymes.[5,6] Cytosolic 3′-AMP-forming enzyme activity in mouse liver is reported to be enhanced by CCl_4 treatment.[7] In contrast, no information is available concerning the intra-mitochondrial localization of 3′-AMP-forming enzyme and the effects of CCl_4 on the intra-mitochondrial enzyme activity. Therefore, it is of interest to know whether or not 3′-AMP-forming enzyme activity in the liver mitochondria or 3′-AMP contents in the liver would be affected by treatment of CCl_4.

In this paper we examined the effect of CCl_4 on 3′-AMP-forming enzyme activity in rat liver. We found that 3′-AMP-forming enzyme activity in rat liver homogenate was increased and the activity in the mitochondria was decreased by CCl_4 treatment.

MATERIALS AND METHODS

Materials —— Poly(A) and 3′-AMP were purchased from Yamasa Shouyu Co. (Chiba, Japan). CCl_4, olive oil, chloroacetaldehyde and the glutamic-pyruvic transaminase-ultraviolet (GPT-UV) test kit Wako were from Wako Pure Chemicals Co. (Osaka, Japan). A high-performance liquid chromatography (HPLC) column (150 × 4.6 mm i.d.) of Chromatorex ODS (DU0005MTP, 5 μm) was kindly supplied by Fuji Silysia Chemical, Ltd. (Aichi, Japan). An anion-exchange resin of Hitachi gel No. 3013-N (5 μm) was kindly supplied by Hitachi (Tokyo, Japan). Other chemicals of reagent grade were obtained commercially. Male Wistar strain rats (6 weeks old) were obtained from Charles River Japan, Inc. (Hino, Japan).

Treatment of Rats with CCl_4 —— CCl_4 was given intraperitoneally as a 40% (v/v) solution in olive oil at a dose of 2 ml/kg body weight. Control rats received the equivalent amount of olive oil intraperitoneally. Animals were fasted for 24 hr during CCl_4 treatment and water was available *ad libitum*. At 24 hr after CCl_4 administration, blood was withdrawn from the abdominal aorta under diethyl ether anesthesia, and the serum was obtained by centrifugation at 1500 × *g* for 10 min. The activity of alanine aminotransferase (ALT) in the serum was measured using a Wako GPT-UV-test kit.

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Table 1. Effect of CCl_4 on Serum ALT Activity, and 3′-AMP and 5′-Adenine Nucleotide Levels in Rat Liver

	ALT	nmol/mg wet weight				
Treatment	IU/l	3′-AMP	AMP	ADP	ATP	Total[#]
None	8.3 ± 0.5	0.032 ± 0.01	1.74 ± 0.08*	2.33 ± 0.23	1.60 ± 0.11	5.67 ± 0.25*
CCl_4	417.9 ± 77.4	ND[##]	1.19 ± 0.12	1.87 ± 0.08	1.38 ± 0.11	4.44 ± 0.22

Each value is expressed as mean ± S.E.M. for five animals. #: AMP + ADP + ATP, ##: not detectable, *$p < 0.01$, significantly different from CCl_4 group.

Preparation of Liver Homogenate —— The liver was quickly removed from CCl_4-treated rat and weighed. The liver (1 g) was homogenized immediately after removal in 19 ml of 20 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA, 0.25 M sucrose and 5 mM 2-mercaptoethanol. The homogenate obtained was kept at –80°C until use, and used as a crude enzyme solution.

Preparation of Crude Mitochondrial Extract from Rat Liver —— Mitochondria were isolated from the liver according to the method by Schneider[8] with a slight modification. Mitochondria thus obtained were washed with 0.1 M phosphate buffer (pH 7.0), and then suspended in 20 mM Tris–HCl buffer (pH 7.5) containing 0.25 M sucrose, 5 mM 2-mercaptoethanol and 1 mM EDTA to make *ca.* 2.5 mg protein per ml. The mitochondria were homogenized with a polytrone homogenizer and then centrifuged at 14000 × *g* for 20 min to separate mitochondrial supernatant and pellet. The pellet was suspended in the same Tris–HCl buffer described above. The obtained supernatant and the pellet suspension were kept at –80°C until use, and used as crude enzyme solutions. Protein was determined by the method of Lowry *et al.*[9] using bovine serum albumin as a standard.

Determination of Adenine Compounds by HPLC —— Adenine compounds in rat liver were extracted using perchloric acid by the method described previously.[3] The acid-soluble compounds were derivatized with chloroacetaldehyde for fluorescence detection.[10] The derivatized adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP) and adenosine 5′-monophosphate (AMP) were analyzed by HPLC using a column (125 × 4.6 mm i.d.) of Hitachi gel No. 3013-N,[10] and the derivatized 3′-AMP and adenosine (Ado) were determined using a column of Chromatorex ODS.[4] The eluate was detected by an Intelligent spectrofluorometer 820-FP (Jasco, Japan).

Determination of 3′-AMP-Forming Enzyme Activity —— 3′-AMP-forming enzyme activity was assessed by determining the enzymic product in the reaction mixture as follows.[4] The reaction mixture (55 μl) consisted of 0.1 M acetate buffer (pH 5.8) containing 20 mM EDTA, 25 μg poly(A) and 5 μl of various enzyme solutions. After incubation of the mixture at 37°C for 60 min, 55 μl of 15 mM $ZnCl_2$ in 0.5 M perchloric acid were added to stop the reaction. The enzymic products were derivatized with chloroacetaldehyde for fluorescence detection.[10] The derivatized compounds were analyzed using a column of Chromatorex ODS maintained at 45°C.[4]

Statistical Analysis —— All data were expressed as the mean ± S.E.M. Statistical evaluation of the data was performed using Student's *t*-test. *p* Values < 0.05 were considered to be significant.

RESULTS

As shown in Table 1, serum ALT activity was greatly enhanced 24 hr after CCl_4 administration. Under these experimental conditions, no 3′-AMP was detected and total amounts of ATP, ADP and AMP in fresh liver from rat fasted for 24 hr were significantly reduced by CCl_4 administration.

Next, the effect of CCl_4 on 3′-AMP-forming enzyme activity in rat liver was examined. As shown in Table 2, the 3′-AMP-forming enzyme activity in the homogenate of CCl_4-treated rat was *ca.* 4-fold higher than that of control. The amounts of 3′-AMP and Ado formed by the mitochondrial supernatant of CCl_4-treated rat were *ca.* 1.5- and 0.4-fold, respectively, compared with those of control. Total amounts of 3′-AMP and Ado formed by CCl_4-treated rat mitochondrial supernatant decreased to *ca.* 50% of control values. Mitochondrial pellets had no 3′-AMP-forming enzyme activity.

DISCUSSION

Intracellular 3′-AMP is classified as a P-site inhibitor of membrane-bound adenylate cyclase.[1] The amount of 3′-AMP (0.032 nmol/mg wet weight) in

Table 2. Effect of CCl_4 on the 3′-AMP-Forming Enzyme Activities in the Homogenate, and Mitochondrial Supernatant and Pellet of Rat Liver

		nmol/60 min/mg protein		
	Treatment	3′-AMP	Ado	3′-AMP + Ado
Homogenate	None	95 ± 15	0	95 ± 15
	CCl_4	378 ± 45	0	378 ± 45
Mitochondrial	None	806 ± 74	51683 ± 7030	52385 ± 7044
supernatant	CCl_4	1201 ± 80*	24179 ± 2014	26827 ± 1798
Mitochondrial	None	ND#	ND	ND
pellet	CCl_4	ND	ND	ND

Each value is expressed as mean ± S.E.M. for five animals. #: not detectable. Ado, Adenosine. *$p < 0.01$, significantly different from none.

the liver of rat fasted for 24 hr (Table 1) was close to the value (0.047 nmol/mg wet weight) reported by Bushfield *et al*.[2] This result indicated that rat liver contains a significant amount of intracellular 3′-AMP which may mediate the physiological function of the cells. Although the pathophysiological role of 3′-AMP is not fully elucidated, taken together with the observation that streptozotocin-evoked augmentation of 3′-AMP content in rat liver could be reversed by insulin treatment,[2] our results which indicate that a decrease in 3′-AMP content occurred in CCl_4-induced hepatitis (Table 1), suggest that formation and accumulation of intracellular 3′-AMP is influenced by the pathophysiological situation.

Although the histochemical localization of 3′-AMP-forming enzyme, a RNase, has not yet been clarified, the enzyme has been shown to exist not only in hepatic mitochondria[3,4] but also in hepatic cytosol.[2,7] In general, RNases do not catalyze release of Ado directly from RNAs. Recently, a molybdate-sensitive acid phosphatase(s), which catalyzed conversion of 3′-AMP to Ado, has been shown to exist in rat liver mitochondria.[4] Therefore, the net 3′-AMP-forming enzyme activity is estimated by the sum of the activity to produce 3′-AMP and Ado. Although the amount of 3′-AMP in hepatic mitochondria treated with CCl_4 was higher than that of control, the decrease in total amount of 3′-AMP and Ado in CCl_4-treated hepatic mitochondria suggested that net 3′-AMP-forming enzyme activity in the mitochondria was largely reduced by treatment with CCl_4 (Table 2).

The toxicity of CCl_4 is known to be mediated by a highly reactive trichloromethyl radical that produce lipid peroxides in hepatocellular membranes.[5,6] The damage to cellular membranes via lipid peroxidation results in leakage of intracellular enzymes. In a previous paper we found that 3′-AMP-forming enzyme activity in the cytosol of mouse liver was enhanced by treatment with CCl_4 and speculated that the increase in activity may result from leakage of the enzyme from mitochondria.[7] However, we had not uncovered the 3′-AMP-forming enzyme activity in mitochondria. The increase in 3′-AMP-forming enzyme activity found in rat cytosol (Table 2) seems to result from leakage of the enzyme from mitochondria via damage to the mitochondrial membrane since the net 3′-AMP-forming enzyme activity was largely reduced by treatment with CCl_4. The change of 3′-AMP-forming enzyme activity in cytosol and mitochondria in rat may affect hepatic functions via modulation of adenylate cyclase and AMP-dependent enzymic reactions.

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