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ACTINOMYCIN D-SENSITIVE INDUCTION OF CHOLINE KINASE BY CARBON TETRACHLORIDE INTOXICATION IN RAT LIVER

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A single intraperitoneal dose(1 ml/kg body weight) of carbon tetrachloride (CCl_4) caused a rapid and drastic induction of choline kinase activity in rat liver cytosol. The administration of either cycloheximide or actinomycin D completely blocked the CCl_4-mediated induction of choline kinase activity, indicating that the elevated activity could be due to the change in the enzyme level. The pretreatment of rats with phenobarbital did not cause any significant effect on hepatic choline kinase induction by CCl_4, suggesting that the induction may not be directly related to the metabolic rate of CCl_4. A considerable part of induced form(s) of choline kinase appeared not to be a form present in the liver of untreated rats. The contribution of adrenals to the CCl_4-mediated hepatic choline kinase induction could be ruled out.

The enzyme choline kinase(CK*)[EC 2.7.1.32] plays an initial role in the synthesis de novo of phosphatidylcholine in all animal tissues by catalyzing the formation of cholinephosphate from free base choline and ATP(1). Recently, the hepatic CK activity has been reported by several laboratories to be inducible in certain physiological states, i.e. treatment of animals(or cells) with excess choline(2), insulin(3), essential fatty acid-deficient diet(4) and a synthetic steroid hormone, diethylstilbestrol(5). On the other hand, we have reported that several polycyclic aromatic hydrocarbon carcinogens such as 3-methylcholanthrene(3-MC) and 3,4-benzo(a)pyrene(well-known inducers of a particular class of microsomal cytochrome P-450(cyt P-450) species) cause a significant elevation of CK activity in rat liver cytosol most likely through a formation

*Abbreviations: CK, choline kinase; 3-MC, 3-methylcholanthrene; cyt P-450, cytochrome P-450; CCl_4, carbon tetrachloride; PB, phenobarbital; TAT, tyrosine aminotransferase; GOT, glutamate-oxaloacetate transaminase; PAGE, polyacrylamide gel electrophoresis.

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of the new enzyme protein(6-7), and proposed that CK induction could be involved in a certain liver intoxication and/or detoxication process(7).

While all of the above findings have suggested that the induction of CK activity may lead to a net increase in de novo phosphatidylcholine biosynthesis, the direct relationship between CK induction and the rate of phosphatidylcholine synthesis has neither been studied(2-4) nor at least clearly elucidated(5,8-9), therefore the physiological meaning of CK induction in these status has still remained obscure.

In this communication, we present a new evidence that CCl_4 intoxication causes a very rapid and drastic induction of CK in rat liver cytosol through an actinomycin D-sensitive mechanism(s). This experimental model could effectively be provided for the future study on the characterization of CK induction and its physiological significance in relation to net phosphatidylcholine biosynthesis in animal liver because of rather well-characterized biochemical sequence of events following CCl_4 intoxication(10).

METHODS

Treatment of Animals: Young male Wistar rats(120-130 g) were used for all experiments. Two rats were housed in one cage in a temperature- and light-controlled room, fed standard laboratory chow and allowed free access to water. CCl_4 was mixed in corn oil(1:1 by vol.) and injected i.p. to the experimental group at a dosage of 1.0 ml/kg body weight. Control animals were given equivalent volumes of corn oil. Injection was performed at 10:00 am after overnight starvation and animals were killed by decapitation at the scheduled time after CCl_4 administration. Food was removed from all cages throughout the experimental period.

Cycloheximide was administered at a single i.p. dose of 2.5 mg/kg in saline 3hr after the CCl_4 injection, while actinomycin D(1 mg/kg in saline) was administered i.p. either at the time of CCl_4 injection or 3hr after the CCl_4. 3-MC was dissolved in hot corn oil and administered at a single i.p. dose of 50 mg/kg 48 hr before the CCl_4 injection, while sodium phenobarbital(PB, 80 mg/kg in saline) was injected i.p. twice 48hr and 24hr before the CCl_4.

Adrenalectomized rats(prepared under pentobarbital anethesia) were used 4 days following surgery with supplementation of 0.9% NaCl in their drinking water.

Enzyme Assays: CK activity in liver 105,000 x g supernatant was assayed as previously described(6,11). Microsomal cyt P-450 content was determined according to the method of Omura and Sato(12). Tyrosine aminotransferase(TAT) [EC 2.6.1.5] was assayed by the method of Granner and Tomkins(13) with aged (4 days at 0°C) liver cytosol in a final incubation volume of 0.93 ml. Plasma GOT(glutamate-oxaloacetate transaminase)[EC 2.6.1.1] activity was determined by Reitman-Frankel's method(14) using S.TA Test kit provided by Wako Pure Chemicals Co. Ltd., Japan.

Native Polyacrylamide Gel Electrophoresis(PAGE) and Sephadex G-200 Gel Filtration: CK was partially purified from both CCl_4-treated(9hr after the injection) and untreated rat liver cytosol through pH 5 precipitation followed by

30-45% saturated $(NH_4)_2SO_4$ fractionation as described elsewhere(11). Approx. 100 μg protein of each preparation was applied on a 7.5% polyacrylamide disk gel (5.5 x 90 mm). Electrophoresis was performed at 3 mA per gel for about 4hr at 4°C, pH 9.3(15). The gel was cut into 3 mm slices after electrophoresis, and incubated overnight at 4°C in the CK assay mixture without [^{14}C]choline and ATP-Mg, then the activity was determined in the presense of [^{14}C]choline and ATP-Mg in a final incubation volume of 0.3 ml. Another portion(0.75 ml, approx. 30 mg protein) of 30-45% $(NH_4)_2SO_4$-precipitated fraction was applied on Sephadex G-200 column(1.5 x 96 cm) previously equilibrated with 20 mM Tris-HCl, pH 7.5 containing 0.1 M KCl and eluted with the same buffer at a flow rate of 3.7 ml/hr. Fraction of 1.0 ml each was collected and assayed for CK activity in a final incubation volume of 0.3 ml.

RESULTS AND DISCUSSION

CK activity is located absolutely in the soluble portion of the liver cell. Fig. 1 shows an induction pattern of hepatic CK activity after a single i.p. dose of CCl_4. After 3hr lag, cytosolic CK activity rapidly increased and reached to the maximum level by 9hr, then gradually declined and returned to the normal level between 24 and 36hr. The decrease of CK specific activity after the peak

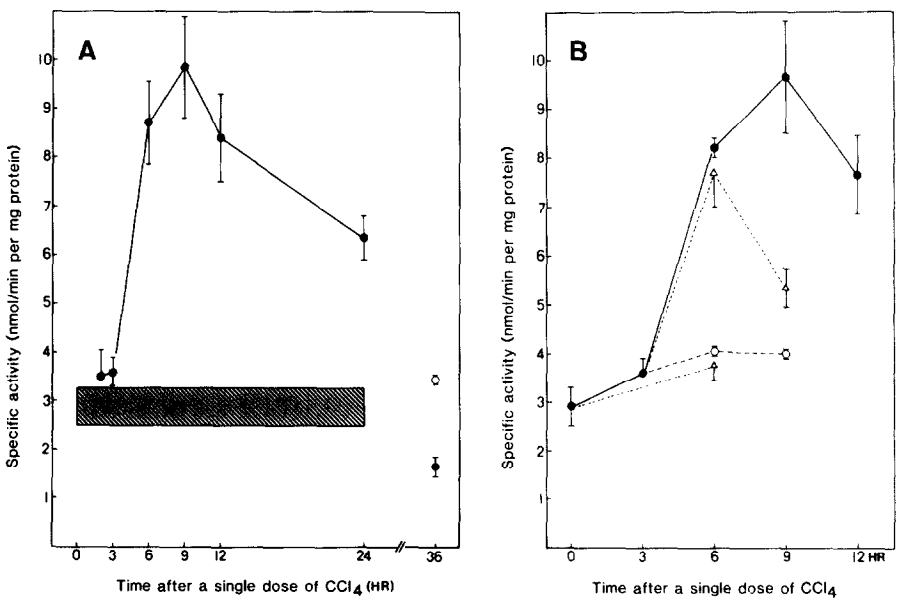

Fig. 1. Induction pattern of CK activity in rat liver cytosol following CCl_4 (1 ml/kg) administration(A) and effect of actinomycin D and cycloheximide on the CCl_4-mediated induction of CK activity(B). The experimental details are described in 'Methods' section. In both A and B, closed circles represent the values from CCl_4-treated group (mean±S.D. of triplicate determinations from 3 to 6 rats). In A, both rectangle with oblique lines and open circle represent the values from untreated control group(mean±S.D. from 3 to 5 rats each point). In B, actinomycin D(Δ----Δ, 1 mg/kg) was injected either at the time of CCl_4 injection or 3hr after the CCl_4 and cycloheximide(o----o, 2.5 mg/kg) was injected 3hr after the CCl_4. Bars are means±S.D. of triplicate determinations from 3 to 4 rats.

around 9hr was probably not due to a rapid turnover of the induced activity but was mainly due to the release of CK activity from the damaged liver cells, because a considerable CK activity was actually detected in blood plasma at the later experimental period, where usually no CK activity is detected.

When actinomycin D was injected to the experimental group at the time of CCl_4 administration, the induction of CK activity by CCl_4 was almost completely blocked as shown in Fig. 1B. When actinomycin D was injected 3hr after the CCl_4, the rapid increase of CK activity in liver cytosol during 3-6hr was not significantly affected. On the other hand, further increase of CK activity during 6-9hr was again considerably blocked by the actinomycin D treatment at 3hr after the CCl_4 administration. These experimental findings strongly suggest that the site of action of CCl_4 leading to the induction of hepatic CK activity could be beyond the transcriptional level. The defect of repression of CK induction during 3-6hr after the CCl_4 administration by actinomycin D, when injected at 3hr point, indicates most likely that messenger for CK, which had previously been formed by CCl_4 treatment, continued as template for additional enzyme synthesis during 3-6hr. An inhibitor of protein synthesis, cycloheximide, blocked almost completely the induction of CK activity by CCl_4 even when it was injected 3hr after the CCl_4 (Fig. 1B). The induction of neither actinomycin D nor cycloheximide caused any significant effect on the basal CK activity in liver cytosol from untreated control rats during the experimental period(data not shown).

Next, in order to know whether the induction of hepatic CK may or may not be related to the magnitude of liver injury by CCl_4, the effect of pretreatment of rats with certain inducers of microsomal drug-metabolizing system on CCl_4-mediated hepatic CK induction was investigated. Several studies(16-19) have provided evidence that PB-type inducers strongly potenciate the CCl_4-mediated liver toxicity via the induction of cyt P-450 species which catalyze a formation of toxic metabolite(s), while another type of cyt P-450 inducer such as 3-MC does not affect the metabolic rate of CCl_4 therefore CCl_4-mediated liver toxicity. When plasma GOT level and hepatic microsomal cyt P-450 content were estimated as parameters of liver injury, the pretreatment of PB actually potenciated CCl_4-

Table I

Effect of 3-MC- or PB-pretreatment on CCl_4-mediated CK induction and other parameters of liver toxicity following CCl_4

Time after CCl_4 administration(hr)	3-MC			PB		
	CK *	GOT**	Cyt P-450***	CK	GOT	Cyt P-450
0	6.41±0.38	137± 16	1.18±0.03	2.77±0.33	115± 7	1.11±0.10
3	6.85	233± 41	0.55±0.11	3.61±0.12	560± 63	0.37±0.05
6	10.03±0.40	322± 26	0.58±0.05	7.93±1.03	940± 92	0.23±0.06
9	12.42±0.37	855± 53		11.33±0.66	955± 82	
12	10.70±1.43	930± 49	0.51±0.01	9.44±0.38	680±143	0.18±0.04
24	7.59±0.37	1035±100	0.35±0.06	4.13±1.10	2420±163	0.12±0.03

*nmol/min/mg protein **Karmen unit ***nmol/mg protein

Treatment of animals and other experimental details are described in 'Methods' section. Values are means±S.D. of triplicate measurements from 3 rats.

mediated hepatotoxicity in our investigation, i.e. an increase in plasma GOT activity and a decrease in cyt P-450 content(Table I). The pretreatment with 3-MC caused no significant change in these parameters when compared to the values from rats treated with CCl_4 alone(data not shown). On the other hand, the induction pattern of hepatic CK activity following CCl_4 was not significantly affected by the PB-pretreatment, indicating that the CCl_4-mediated CK induction in rat liver could not be directly related to the metabolism of CCl_4 itself. While the treatment of rats with 3-MC alone did cause approx. 2-fold induction of hepatic CK activity, as previously reported(6-7), further induction of CK was found to occur following CCl_4 administration. This finding probably suggests that there exists a different mechanism between 3-MC and CCl_4 by which cytosolic CK activity is induced in rat liver.

On the other hand, the blood levels of both catecholamines(20) and glucocorticoids(21) have been reported to increase in an early stage of CCl_4 intoxication. Glucocorticoid-mediated activation of CK has also been reported for rat mammary gland in organ culture(22). Therefore, the possibility of the contribution of adrenals to the hepatic CK induction by CCl_4 was examined with adrenalectomized rats. As shown in Table II, the induction of hepatic CK activity following CCl_4 was not essentially affected by adrenalectomy, although the basal CK activity was found to decrease in the adrenalectomized group. The very similar results

Table II

Effect of adrenalectomy on CCl_4-mediated induction of CK and TAT activities in rat liver cytosol

	CK	TAT
Treatment (N)	nmol/min/mg	μmol/min/mg
Sham-operated (3)	2.19±0.09	15.4±1.5
Sham-operated + CCl_4 (4)	7.57±0.34	22.8±3.0
Adrenalectomized (5)	1.65±0.14	7.7±0.9
Adrenalectomized + CCl_4 (4)	6.77±0.85	17.8±4.1

Treatment of animals and other experimental details are described in 'Method' section. Values are means±S.D. of triplicate determinations from the indicated number of animals in parenthesis.

were obtained for the activation of hepatic TAT which is known as one of the most typical enzyme under the control of glucocorticoids. These findings indicate that the participation of adrenals could be ruled out with respect to the CCl_4-mediated induction of both CK and TAT activities in rat liver cytosol.

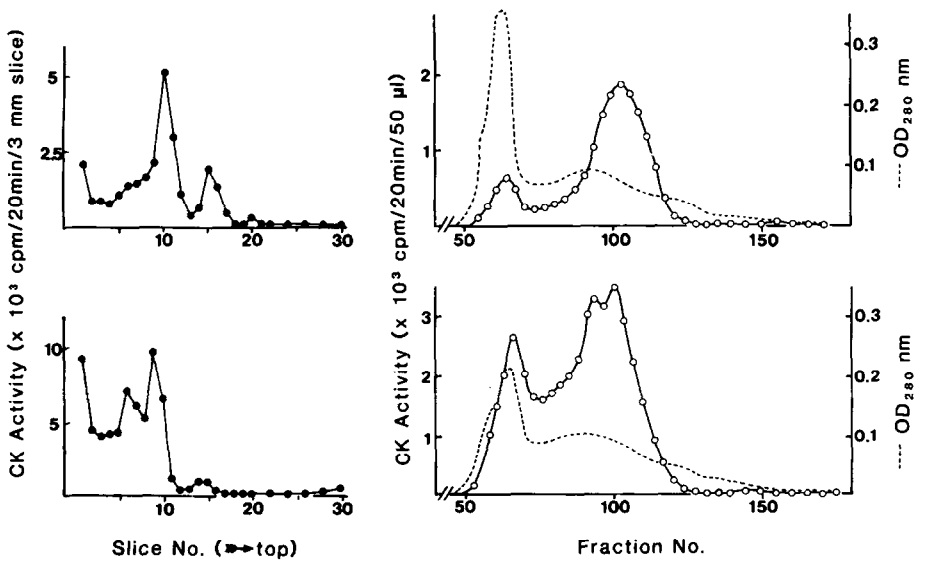

Fig. 2. Comparison of native PAGE(left) and Sephadex G-200 gel chromatographic (right) patterns of CK activity between the preparations from untreated(upper) and CCl_4-treated(lower) rat liver cytosol. The experimental details are described in 'Methods' section. The CK assay mixture contained 0.1 M Tris-HCl, pH 8.75, 10 mM ATP-2Na, 10 mM $MgCl_2$, [Me-^{14}C]choline chloride(spec. act. 0.7Ci/mol) and either 3 mm gel slice after PAGE or 50 μl of the fraction after gel filtration, in a final volume of 0.3 ml. Incubations were carried out at 37°C for 20min. The CK activity was denoted as ^{14}C cpm incorporated into cholinephosphate during the incubation.

Finally, CK was partially purified from liver 105,000 x g supernatant through acid treatment followed by 30-45% saturated $(NH_4)_2SO_4$ fractionation, then both electrophoretic and gel chromatographic patterns of CK activity were compared between the control and CCl_4-induced preparations(Fig. 2). The data show that there exist several forms of CK in rat liver cytosol, as reported by Brophy et al.(23), and the treatment of rats with CCl_4 resulted in the induction of a relatively larger molecular form(s) of CK which was at least not a predominant form in the liver of untreated control rats. More precise experiments, however, will be needed for the characterization of the induced enzyme as well as the complete purification of each form of CK protein and these lines of experiment are now actually in active progress in this laboratory.

The overall results strongly suggest that CK induction is involved in the early process of liver intoxication by CCl_4, supposedly as a protection mechanism of liver cells against highly toxic invaders like CCl_4.

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