Biochemical Pharmacology, Vol. 31, No. 17, pp. 2837-2840, 1982.
Printed in Great Britain.

0006-2952/82/172837-04$03.00/0

PRELIMINARY COMMUNICATIONS

EFFECT OF CARBON TETRACHLORIDE-INDUCED SOLUBLE PROTEIN ON MICROSOMAL NADPH OXIDASE ACTIVITY OF RAT LIVER

Tohoru Hasegawa,* Masana Ogata and Katsumaro Tomokuni*

Department of Public Health, Okayama University Medical School, Okayama 700, Japan

(Received 19 April 1982; accepted 18 May 1982)

Liver microsomes are capable of oxidizing NADPH at slow rates (1). This communication describes evidence that a soluble protein extracted from liver cytoplasm of rats that were intoxicated with CCl_4 stimulates microsomal NADPH oxidation.

CCl_4 (0.5 ml/kg of body weight) was injected into Donryu male rats (200-250 g body weight) intraperitoneally. Six days after the CCl_4 injection, livers were perfused with 0.25 M sucrose, 4 mM Tris-HCl buffer (pH 7.5). Then a 20% liver homogenate was made in 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5). It was centrifuged at 10,000g for 20 min, and microsomes were then sedimented from the supernatant by centrifuging it at 105,000g for 60 min. The sedimented microsomes were resuspended in 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5) and used in this experiment as CCl_4 microsomes. The resulting 105,000g supernatant was saved for extraction of CCl_4 soluble protein. Normal microsomes and normal 105,000g supernatant that was saved for extraction of normal soluble protein were also extracted from normal rat liver.

Crude extracts of CCl_4, and normal, soluble protein were made by ammonium sulfate precipitation at 4° with stirring. Saturated ammonium sulfate (of which the pH was adjusted by Tris to 7.5) was added gradually to the CCl_4 or normal, 105,000g supernatant until the supernatant solution was 40% saturated. The solution was centrifuged and the supernatant was saved. To obtain 40-70% saturation, the ammonium sulfate content of the 40% saturated supernatant solution was raised to 70% saturated. The solution was then

---

*Present address: Department of Community Health Science, Saga Medical School, Nabeshima, Saga 840-01, Japan.

centrifuged at 10,000g for 10 min, the precipitate was redissolved in 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5), and the solution was dialyzed overnight at 4° to remove ammonium sulfate. The precipitate that was obtained between 40 and 70% ammonium sulfate saturation was used in this experiment as a CCl_4 soluble protein or a normal soluble protein.

Figure 1 shows that NADPH was aerobically oxidized at pH 7.5 in the presence of CCl_4 microsomes (2-4 nmoles/min/mg protein at 20°). On addition of normal soluble protein (3.5 mg protein/ml), this slow oxidation was stimulated 3- to 5-fold. This stimulation of oxidation was more significant on addition of CCl_4 soluble protein (same protein concentration as normal soluble protein control). The CCl_4 soluble protein stimulated oxidation 20- to 25-fold. In the presence of normal microsomes, NADPH was oxidized faster than in the presence of CCl_4 microsomes (4-6 nmoles/min/mg protein at 20°). On addition of normal soluble protein, this NADPH oxidation was stimulated 2- to 3-fold; on addition of CCl_4 soluble protein, NADPH oxidation was stimulated 5- to 7-fold. The stimulated NADPH oxidation induced by the addition of CCl_4 soluble protein was faster in the presence of CCl_4 microsomes than in the presence of normal microsomes.

The 40-70% ammonium sulfate precipitate was heated at 100° for 10 min; the boiled precipitate had no stimulating effect on NADPH oxidation, which is consistent with a protein effect.

The mechanism of stimulation of microsomal NADPH oxidation induced by a CCl_4 soluble protein is not yet accounted for. The aerobic oxidation of NADPH stimulated by normal, or CCl_4, soluble protein was not inhibited by cyanide (final concentration 1 mM), indicating that the stimulation did not involve a cyanide sensitive component, such as cytochrome oxidase (1). On the contrary, carbon monoxide slowed the velocity of oxidation induced by the CCl_4 soluble protein by 50%. However, carbon monoxide did not slow the velocity of oxidation induced by normal soluble protein, that is, the effect of carbon monoxide on the stimulation of NADPH oxidation by CCl_4 soluble protein was not the same as that on the stimulation by normal soluble protein, suggesting that the stimulation on NADPH oxidation induced by CCl_4 soluble protein involved carbon monoxide sensitive components, such as hemoprotein (2), but that the stimulating reaction induced by normal soluble protein did not.

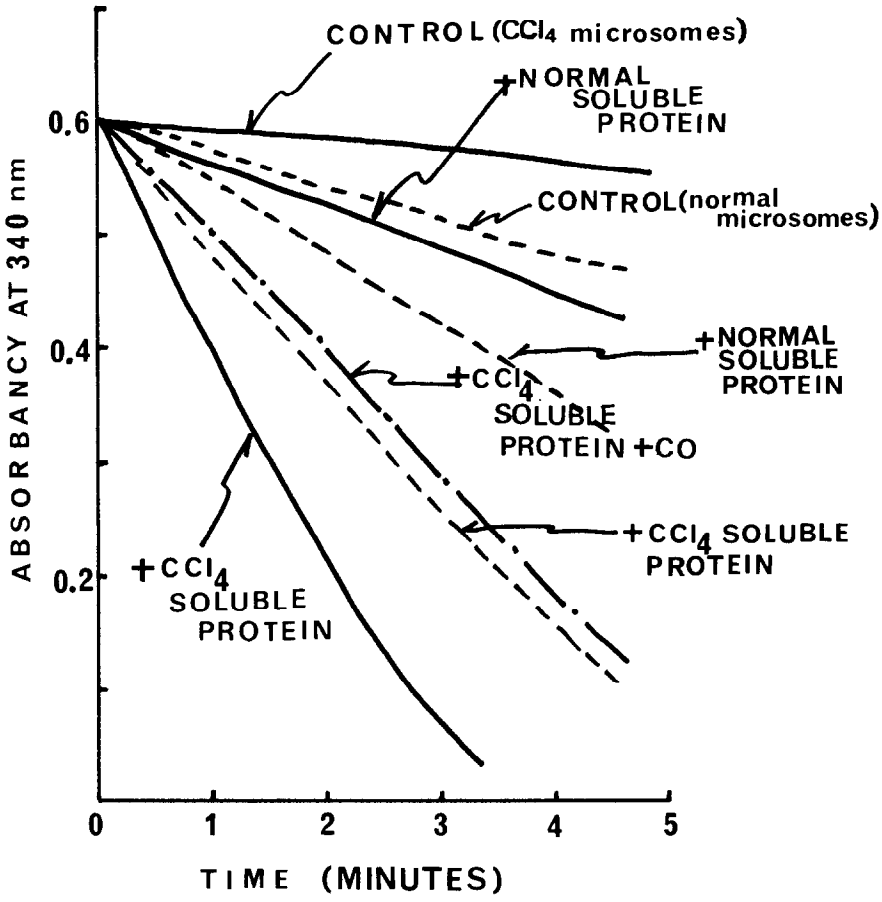

Fig. 1. Effect of normal, or CCl_4, soluble protein on NADPH oxidation by liver microsomes under aerobic conditions. The final reaction mixture contained CCl_4 or normal microsomes (2.6 mg protein/ml), 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5), 0.1 mM NADPH and normal or CCl_4 soluble protein (3.5 mg protein/ml) in a total volume of 4.0 ml. The changes in absorbancy at 340 nm were recorded in a Hitachi two-wavelength double beam difference recording spectrophotometer. Temperature was 20°. CO was bubbled through the reaction mixture for 30 sec (one bubble/sec). Key: (——) in the presence of CCl_4 microsomes; (·····) in the presence of normal microsomes; (— · —) in the presence of CCl_4 microsomes + CCl_4 soluble protein + CO.

These results may be associated with some processes of recovery from the impairment by CCl_4 of microsomal electron transport, because the stimulation of CCl_4 protein was increased with increasing time after the CCl_4 injection.

REFERENCES

1. J.R. Gillette, B.B. Brodie and B.N. La Du, J. Pharmac. exp. Ther. 119, 532 (1957).

2 T. Omura, R. Sato, D.Y. Cooper, O. Rosenthal and R.W. Estabrook, Fedn Proc. 24, 1181 (1965).