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INDUCTION OF CHOLINE KINASE BY POLYCYCLIC AROMATIC HYDROCARBON CARCINOGENS IN RAT LIVER

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SUMMARY

The administration to rats of polycyclic aromatic hydrocarbons such as 3-methylcholanthrene, 3,4-benzo(a)pyrene and β-naphthoflavone caused a significant elevation of hepatic choline kinase activity. On the other hand, phenobarbital-type inducers(phenobarbital, 1,1,1-trichloro 2,2-bis(p-chlorophenyl) ethane(DDT) and hexachlorobenzene) did not stimulate the activity at all. The administration of either cycloheximide or actinomycin D completely depressed the elevation of choline kinase activity induced by polycyclic aromatic hydrocarbons, indicating that the elevated activity by these chemicals could be due to the change in the enzyme level. These results strongly suggest that induction of choline kinase are involved in the sequence of events leading to the induction of hepatic drug metabolism by polycyclic aromatic hydrocarbons.

INTRODUCTION

It has been known for quite some time that a wide variety of drugs, carcinogens, other environmental chemicals and steroids induce their metabolism and/or that of other compounds via induction of cytochrome P-450, a key enzyme of microsomal mixed-function oxygenase system(1,2). PB and 3-MC have been proposed to represent two prototype inducers, the former inducing the cytochrome P-450 species and the latter, so-called cytochrome P-448 species(3,4). More species of this cytochrome have been reported(5-11). Although several laboratories have made considerable progress toward the purification and characterization of multiple forms of cytochrome P-450(12-17), the difference in the mechanism of induction process by individual inducer has not been fully understood.

Abbreviations: PB, phenobarbital; 3-MC, 3-methylcholanthrene; β-NF, β-naphthoflavone; 3,4-BP, 3,4-benzo(a)pyrene; PCBs, polychlorinated biphenyls; DDT, 1,1,1-trichloro 2,2-bis(p-chlorophenyl)ethane; HCB, hexachlorobenzene.

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On the other hand, the fact that most of these inducers are highly lipophilic and expected to interact with intracellular membrane components, has led us to study the effect of administration of the typical inducers on membrane lipid metabolism(18,19). We have found that there exists a possible relationship between induction of microsomal drug-metabolizing system and modulation of phospholipid metabolism, especially of phosphatidylcholine biosynthesis in rat liver(19,20). One of the most striking differences in the effect of PB and 3-MC on phosphatidylcholine metabolism was seen toward the activity of choline kinase[EC 2.7.1.32], a first key enzyme in phosphatidylcholine biosynthesis; 3-MC caused a significant increase in choline kinase activity while PB decreased the activity(20).

In the present study, we examined what a mechanism may be involved in the stimulation of choline kinase activity after the administration of 3-MC-type inducers, together with the time course comparison of the induction process between cytochrome P-448 and choline kinase activity in rat liver.

METHODS

Inducer Treatment of Animals: Young Wister male rats(100-120 g) were used for all experiments. 2 rats were housed in one cage each, fed standard laboratory chow and allowed free access to water. 3-MC, β-NF, 3,4-BP, PCBs(Polychloro Biphenyl, tetra, from Wako Pure Chemical Industries Co. Ltd., Japan) and DDT were dissolved in hot corn oil while HCB was dispersed in corn oil under brief sonication. PB(Na salt) was dissolved in saline. All chemicals were injected i.p. to the experimental group at a dosage of 50 mg(3-MC, 3,4-BP), 40 mg(β-NF), 30 mg(DDT), 80 mg(PB) and 100 mg(PCBs, HCB) per kg, respectively. Control animals were given equivalent volumes(0.25 ml per 100 g body wt.) of vehicle. Injection was performed at 18:00 otherwise indicated and animals were killed 16 hr after the injection, i.e. at 10:00 in the next morning. Food was removed from the cage throughout experimental period. Cycloheximide was administered at a single i.p. dose of 2.5 mg per kg in saline at the time of 3-MC injection, while actinomycin D(1 mg per kg in saline, i.p.) was administered twice 1 hr before and 6 hr after the 3-MC injection. In the time course study, the experiment was started at 10:00 by a single i.p. dose of 3-MC injection. Food was removed for the first 24 hr and every night-time thereafter(22:00 through 10:00 in the next morning).

Assay for Choline Kinase and Cytochrome P-450: A 20%(W/V) homogenate of liver was prepared in 1.15%(W/V) KCl containing 10 mM Tris-HCl, pH 7.4, using a Teflon pestle homogenizer. The homogenate was centrifuged twice at 15,000 x g for 15 min and the supernatant was centrifuged at 105,000 x g for 60 min. The resulting supernatant was used for choline kinase assay. The microsomal pellet was rehomogenized in a proper amount of isotonic KCl and recentrifuged at

105,000 x g for 30 min. The washed microsomes were used for cytochrome P-450 determination according to the method of Omura and Sato(21). Choline kinase activity was assayed by a slight modification of the method of Weinhold and Rethy(22) as described elsewhere(20).

RESULTS AND DISCUSSION

Choline kinase is located in the soluble portion of the liver cell. The reaction rate was constant for 30 min and proportional to the amount of enzyme preparation within the range of 0.6 mg protein per incubation.

The induction patterns of both choline kinase activity and microsomal cytochrome P-450(P-448) up to 96 hr after a single administration of 3-MC are compared in Fig. 1, where the activity and the cytochrome content are represented on the basis of mg protein(A and C) and whole liver(B and D), respectively. The activity of choline kinase showed a marked decrease at 12 hr in both control and experimental groups probably due to its diurnal rhythm. This decrease in control animals was partly reversed at the later periods where the activity was assayed in the morning. However, the later activities in the control group were kept relatively low when compared to that of 0-time control, indicating that overnight fasting resulted in a diminished activity of choline kinase. Recently, Groener et al.(23) also reported that fasting caused a decrease in choline kinase activity in rat liver. The administration of 3-MC resulted in a considerable increase in choline kinase activity. This stimulatory effect by 3-MC was significant as early as 6 hr after the administration, reached to the maximum level (100% increase) by 48 hr and was sustained at 96 hr with either representation of the activities(Fig. 1A and B). On the other hand, the induction of cytochrome P-450(P-448) became significant 12 hr after the 3-MC administration, reached to the maximum at 72 hr, then gradually decreased, as was clearly shown in Fig. 1C and D. The overall patterns of induction by 3-MC administration of both cytosolic choline kinase activity and microsomal cytochrome P-448 in rat liver were very similar to each other when reffered to the function of time, suggesting that the increase in choline kinase activity may be involved in the mechanism of induction by 3-MC of hepatic microsomal mixed-function oxygenase system.

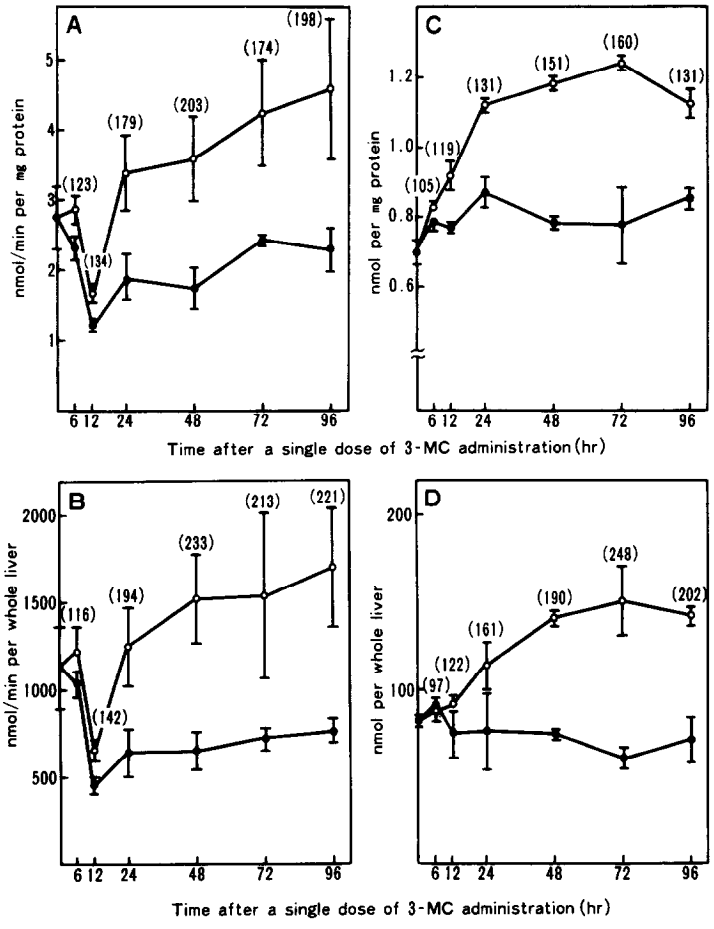

Fig. 1. Induction patterns of cytosolic choline kinase activity(A and B) and microsomal cytochrome P-450(C and D) in rat liver following 3-MC administration. The experimental details are described in 'Methods' section. Open and closed circles represent the values from 3-MC-treated and control animals, respectively. Bars are means ± S.E. of triplicate measurements from three animals. The number in parenthesis represents % of control value(average).

In order to clarify the nature of the increase in choline kinase activity in rat liver by 3-MC administration, several experiments were performed, in which a well-known inhibitor of protein synthesis(cycloheximide) or mRNA synthesis(actinomycin D) was injected to both control and experimental animals. As shown in Table I, either of the inhibitors completely blocked the 3-MC-mediated increase in choline kinase activity in liver 105,000 x g supernatant fraction, indicating that 3-MC could elevate a formation of the new enzyme protein, and

TABLE I

Effect of cycloheximide and actinomycin D on 3-MC-mediated increase in choline kinase activity in rat liver

	Choline kinase activity	
	nmol/min per mg protein	nmol/min per whole liver
Expt. 1.		
Control	2.36 ± 0.36	722 ± 70
Cycloheximide	1.95 ± 0.36	600 ± 28
3-MC	3.87 ± 0.65*	1153 ± 83*
3-MC + Cycloheximide	2.27 ± 0.32	707 ± 50
Expt. 2.		
Control	2.24 ± 0.23	643 ± 47
Actinomycin D	2.37 ± 0.15	670 ± 35
3-MC	3.62 ± 0.69*	1075 ± 205*
3-MC + Actinomycin D	2.24 ± 0.36	646 ± 102

Treatment of animals and other experimental details are described in 'Methods' section. Values are means ± S.E. of triplicate measurements from four animals.
* Significantly ($p < 0.01$) different from untreated control values.

that the site of action of 3-MC leading to the induction of choline kinase synthesis could be beyond the transcriptional level. The former possibility has also been supported by the following criteria(data not shown); 1) the in

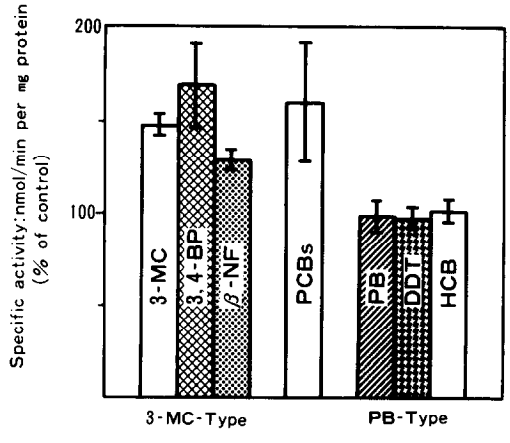

Fig. 2. The effect of different types of inducers of microsomal mixed-function oxygenase system on cytosolic choline kinase activity in rat liver. Treatment of animals and other experimental details are described in 'Methods' section. Bars represent means ± S.E. of triplicate measurements from three or four rats.

vitro addition of 3-MC to the enzyme preparation did not stimulate choline kinase activity at all. 2) a degree of increase in the specific activity(nmol/min per mg protein) following 3-MC administration was much higher when compared with the partially purified choline kinase preparation(through 30-45% ammonium sulphate precipitation, DEAE-cellulose column chromatography).

Next, to define whether the induction of choline kinase is specific for 3-MC or a general event for polycyclic aromatic hydrocarbons, i.e. cytochrome P-448 inducers, the effect of well-known inducers of microsomal mixed-function oxygenase system on choline kinase activity was investigated. As shown in Fig. 2, it was clearly demonstrated that the induction of choline kinase in rat liver is a very common event for so-called cytochrome P-448 inducers including PCBs, which have been proposed to be a mixed type of inducer of both PB and 3-MC(24-27).

The overall results strongly suggest that the induction of choline kinase are involved in the mechanism of induction of microsomal mixed-function oxygenase system caused by 3-MC-type but not by PB-type inducers in rat liver. The physiological significance of choline kinase induction by polycyclic aromatic hydrocarbon carcinogens in relation to hepatic phosphatidylcholine metabolism is now under investigation in this laboratory.

REFERENCES

1. Conney, A.H. (1967) Pharmacol. Rev., 4, 317-366.
2. Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R.W., and Conney, A.H. eds (1977) Microsomes and Drug Oxidations, Pergamon Press, Oxford.
3. Sladek, N.E. and Mannering, G.J. (1966) Biochem. Biophys. Res. Commun., 24, 668-674.
4. Mannering, G.J., Sladek, N.E., Parli, C.J. and Shoeman, D.W. (1969) in Microsomes and Drug Oxidations, (Gillette, J.R., Conney, A.H., Cosmides, G.J., Estabrook, R.W., Fouts, J.R. and Mannering, G.J. eds) pp. 303-330.
5. Haugen, D.A., van der Hoeven, T.A. and Coon, M.J. (1975) J. Biol. Chem., 250, 3567-3570.
6. Welton, A.F., O'Neal, F.O., Chaney, L.C. and Aust, S.D. (1975) J. Biol. Chem., 250, 5631-5639.
7. Gibson, G.G. and Schenkman, J.B. (1978) J. Biol. Chem., 253, 5957-5963.
8. Agosin, M., Morello, A., White, R., Repetto, Y. and Pedemonte, J. (1979) J. Biol. Chem., 254, 9915-9920.
9. Negishi, M. and Nebert, W. (1979) J. Biol. Chem., 254, 11015-11023.
10. Elshourbagy, N.A. and Guzelian, P.S. (1980) J. Biol. Chem., 250, 1279-1285.

11. Sugiyama, T., Miki, N. and Yamano, T. (1980) J. Biochem., 87, 1457-1467.
12. Imai, Y. and Sato, R. (1974) Biochem. Biophys. Res. Commun., 60, 8-14.
13. Hashimoto, C. and Imai, Y. (1976) Biochem. Biophys. Res. Commun., 68, 821-827.
14. Haugen, D.A. and Coon, M.J. (1976) J. Biol. Chem., 251, 7929-7939.
15. Guengerich, F.P. (1978) J. Biol. Chem., 253, 7931-7939.
16. Botelho, L.H., Ryan, D.E. and Levin, W. (1979) J. Biol. Chem., 254, 5635-5640.
17. Masuda-Mikawa, R., Fujii-Kuriyama, Y., Negishi, M. and Tashiro, Y. (1979) J. Biochem., 86, 1383-1394.
18. Ishidate, K. and Nakazawa, Y. (1976) Biochem. Pharmacol., 25, 1255-1260.
19. Ishidate, K., Yoshida, M. and Nakazawa, Y. (1978) Biochem. Pharmacol., 27, 2595-2603.
20. Ishidate, K., Tsuruoka, M. and Nakazawa, Y. (1980) Biochim. Biophys. Acta, in press (Lipids and Lipid Metabolism).
21. Omura, T. and Sato, R. (1964) J. Biol. Chem., 239, 2370-2378.
22. Weinhold, P.A. and Rethy, V.B. (1974) Biochemistry, 13, 5135-5141.
23. Groener, J.E.M., Klein, W. and van Golde, L.M.G. (1979) Arch. Biochem. Biophys., 198, 287-295.
24. Arvares, A.P. and Kappas, A. (1977) J. Biol. Chem., 252, 6373-6378.
25. Yoshimura, H., Ozawa, N. and Saeki, S. (1978) Chem. Pharm. Bull., 26, 1215-1221.
26. Goldstein, J.A., Hickman, P., Bergman, H., McKinney, J.D. and Walker, M.P. (1977) Chem.-Biol. Interact., 17, 69-87.
27. Poland, A. and Glover, E. (1977) Mol. Pharmacol., 13, 924-938.