Vol. 107, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1982 Pages 6-11

3-METHYLCHOLANTHRENE INDUCES PHENOBARBITAL-INDUCED CYTOCHROME P-450 HEMOPROTEIN IN FETAL LIVER AND NOT CYTOCHROME P-448 HEMOPROTEIN INDUCED IN MATERNAL LIVER OF RATS

K. Mizokami, K. Inoue, M. Sunouchi, K. Fujimori, A. Takanaka and Y. Omori

Division of Pharmacology, Biological Safety Research Center,

National Institute of Hygienic Sciences,

1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan

Received May 4, 1982

Summary: The characteristic nature of the drug-metabolizing system in fetal liver microsomes of rats was investigated. The aminopyrine(AM)- and the hexobarbital(HB)-metabolizing activities in fetal liver microsomes of the 21st day of pregnancy were induced by the maternal administration of 3-methylcholanthrene (3-MC) once daily on the 18th and the 19th day of pregnancy, while they were inhibited in maternal liver microsomes. The inductions of the AM- and the HB-metabolizing enzymes in fetal liver microsomes of rat by the maternal administration of 3-MC occurred exclusively in fetal period and simultaneously hemoprotein like phenobarbital-induced type P-450 different from that in maternal liver microsomes was newly induced in fetal liver microsomes of rats.

INTRODUCTION

It is well established that activities of drug-metabolizing enzymes and cytochrome P-450 contents in fetal liver microsomes of rats are very low(1-3). Most of the studies on the drug-metabolizing system in fetal rat have been concerned with the induction of the drug-metabolizing activities and cytochrome P-450 levels by the maternal administration of various inducers(4-6). The nature of the drug-metabolizing system in fetal liver microsomes of rat have not been well examined. We have been engaged in the investigation of the drug-metabolizing system in fetal liver of rat(7). Previously, we have obtained the results that aminopyrine(AM)- and the hexobarbital(HB)-metabolizing activities in fetal liver microsomes of the 21st day of pregnancy were

Abbreviations used are: AM, aminopyrine, HB, hexobarbital, 3-MC, 3-methylcholanthrene, PB, phenobarbital, CO, carbon monoxide, EtCN, ethylisocyanide, i.p., intraperitoneally.

0006-291X/82/130006-06$01.00/0

induced by the maternal administraiton of 3-methylcholanthrene(3-MC) once daily on the 18th and the 19th day of pregnancy. In addition, administration of 3-MC to newborn rats once daily on the 0th and the 1st day of age inhibited the AM- and the HB-metabolizing activities in liver microsomes of 3 day-old neonates(Sunouchi et al., manuscript in preparation). Based on these results, the characteristic nature of the drug-metabolizing system in fetal liver microsomes of rats was investigated.

MATERIALS AND METHODS

Animals and treatments Experimental animals used were Wistar strain rats weighing 250-350 g. Five females were mated with two males overnight. The next day was considered as day 0 of pregnancy and the birthday was considered as day 0 of age. 3-MC(25 or 40 mg/kg) dissolved in olive oil and phenobarbital(PB) sodium(60 mg/kg) dissolved in normal saline were intraperitoneally(i.p.) administered.

Preparation of liver Both the maternal and the fetal or neonatal livers pooled by litter were homogenized with 3 vol. of 1.15 % KCl solution and centrifuged at 10,000 x g for 20 min. For the preparation of microsomal fractions, the resulting supernatant fractions were then centrifuged at 105,000 x g for 60 min. Microsomal precipitates were resuspended in 1.15 % KCl solution to adjust the concentration of 250 mg liver/ml(7).

Assays of drug-metabolism The determinations of the AM- and the HB-metabolizing activities were performed by a slight modification of the method previously reported(7). The contents of cytochrome P-450 in maternal liver microsomes were determined by the method of Omura and Sato(9). The contents of cytochrome P-450 in fetal and neonatal liver microsomes were determined by the method of Johannessen and DePierre(10). The protein contents of the 10,000 x g supernatant and microsomal suspensions were determined by the method of Lowry et al(16).

Preparation of the microsomal fraction through Sepharose 2B and characterization of spectral properties of cytochrome P-450 For the characterization of the induced cytochrome P-450 in fetal and maternal liver microsomes of the 21st day of pregnancy, each microsomal fraction was prepared according to the method of Tangen et al(11). The carbon monoxide(CO)-difference spectra of cytochrome P-450 were measured in a microsomal suspension containing 1 mg protein/ml as discribed by Omura and Sato(9). The ethylisocyanide(EtCN)-difference spectra were determined in a similar manner that EtCN(final concentration, 10 mM) was used as the ligand as previously reported(12). Data was presented as the ratio of 455/430 nm(the differences between absorption at 429-490 nm and 450-490 nm were used as estimates of the 430 and 455 peaks, respectively). The diethylphenylphosphine-difference spectra were also determined as described previously using 0.1 M Tris/HCl-buffer pH:7.6 as a suspension buffer and diethylphenylphosphine(final concentration, 1.5 mM) as the ligand(15) Protein was measured by the method of Lowry et al(16). All the spectral determinations were performed on a Union SM-401 spectrophotometer using 1-cm^2 cuvettes.

RESULTS AND DISCUSSIONS

The first experiment was designed to examine the dose-response for the inductions of the AM- and the HB-metabolizing activities in fetal liver microsomes by the maternal administration of 3-MC. 3-MC was administered(i.p.) to

Table 1 Effect of the maternal administration of 3-methylcholanthrene on the drug-metabolizing activities in fetal and maternal liver microsomes of rats

Source of livers	Treatment	AM-metabolizing activities (nmoles/mg protein/30 min)	HB-metabolizing activities (nmoles/mg protein/30 min)	P-450 hemoprotein (nmoles/mg protein)
Fetal rat	None	1.71 ± 0.09	0.87 ± 0.06	0.042 ± 0.002
	3-MC(25 mg/kg)	2.53 ± 0.16**	1.31 ± 0.10**	0.136 ± 0.011**
	3-MC(40 mg/kg)	3.79 ± 0.36**	2.11 ± 0.34**	0.160 ± 0.020**
Maternal rat	None	37.75 ± 1.11	25.81 ± 1.04	0.488 ± 0.009
	3-MC(25 mg/kg)	33.46 ± 1.75*	22.90 ± 1.02*	0.880 ± 0.019**
	3-MC(40 mg/kg)	35.14 ± 2.16	24.40 ± 0.12	0.862 ± 0.071**

3-MC(25 or 40 mg/2 ml of olive oil/kg of body weight, i.p.) was administered to pregnant rats once daily on the 18th and the 19th day of pregnancy. For control, olive oil was administered only. Pregnant rats were sacrificed on the 21st day of pregnancy. Both maternal and fetal livers were immediately excised. Each value is mean ± s. e. An asterisk indicates a significant difference from control(* $P<0.05$, ** $P<0.01$).

pregnant rats on the 18th and the 19th day of pregnancy. As shown in Table 1, the AM- and the HB-metabolizing activities in fetal liver microsomes of the 21st day of pregnancy were induced and the extents of the inductions were dose-dependent, while any inductive effect on them was not observed in maternal liver microsomes. The AM- and the HB-metabolizing activities are known to be inhibited in rats treated with 3-MC(8). In contrast, it is worth noting phenomena that the AM- and the HB-metabolizing activities were induced in fetal liver microsomes by the maternal administration of 3-MC. The present results, together with the findings so far reported, suggest that the inductions of the AM- and the HB-metabolizing activities occur only during fetal period. In this respects, second experiments were performed.

3-MC was administered(i.p.) to the pregnant rats on the 19th and the 20th day of pregnancy and the inductive effects on the AM- and the HB-metabolizing activities were examined to ascertain whether the inductive effects would be observed in newborn liver microsomes immediately after birth. As presented in Table 2, any inductive effect of 3-MC on the AM- and the HB-metabolizing activities was not observed in newborn liver microsomes although 3-MC was administered during fetal period. The disappearance of the inductive effect of

Table 2 Effect of the maternal administration of 3-methylcholanthrene on the drug-metabolizing activities in liver microsomes of newborn rats

Source of livers	Treatment	AM-metabolizing activities	HB-metabolizing activities	P-450 hemoprotein
		(nmoles/mg protein/30 min)		(nmoles/mg protein)
Newborn rat	None	1.70 ± 0.17	4.85 ± 0.33	0.112 ± 0.007
	3-MC(25 mg/kg)	1.50 ± 0.18	4.58 ± 0.38*	0.170 ± 0.010**

3-MC(25 mg/2 ml of olive oil/kg of body weight, i.p.) was administered to pregnant rats once daily on the 19th and the 20th day of pregnancy. Immediately after parturition, the newborn rats were sacrificed and livers were excised. Each value is mean ± s. e. An asterisk indicates a statistically significant difference from control(* $P<0.05$, ** $P<0.01$).

3-MC on the AM- and the HB-metabolizing activities after birth suggests that hemoprotein different from that induced in maternal liver microsomes is induced in fetal liver microsomes by the maternal administration of 3-MC.

The contamination by hemoglobins and the low levels of cytochrome P-450 make it difficult to characterize the spectral properties of the cytochrome P-450 induced in the microsomes of fetal liver obtained by conventional differential centrifugation. Thus, we prepared liver microsomes by passing 10,000 x g supernatant fraction through Sepharose 2B column to rule out hemoglobin(11). The results summarized in Table 3 clearly indicate that the hemoprotein induced in fetal liver microsomes by the maternal administration of 3-MC is different from the hemoprotein induced in maternal liver microsomes. Surprisingly the maximum of CO-difference spectra of 3-MC-induced cytochrome P-450 induced in fetal liver microsomes was at 450 nm. Cytochrome P-450 from 3-MC-pretreated maternal liver, however, yielded the peak at 448 nm. In addition, cytochrome P-450 from PB-pretreated maternal liver yielded the peak at 450 nm. The hemoprotein induced in fetal liver microsomes by the maternal administration of 3-MC appeared to be the same hemoprotein induced in maternal liver microsomes pretreated with PB. This finding prompted us to characterize the property of the hemoprotein induced in fetal liver microsomes by the administration of 3-MC using EtCN as the ligand. As shown in Table 3, treatment with 3-MC resulted in a marked increase in the ratio of 455/430 nm of the difference spectra of hemoprotein induced in maternal liver microsomes. In contrast, with PB-induced

Table 3 Spectral properties of microsomal P-450 hemoprotein: the maxima correspond to the absorption peaks in the difference spectra(nm)

Ligand	PB-treated maternal liver	3-MC-maternal-treated fetal liver	3-MC-treated maternal liver
Carbon monoxide	450	450	448
Ethylisocyanide	429, 454	429, 454	430, 454
(Ratio of 455/430)	0.40	0.42	1.23
Diethylphenylphosphine	459	458	–

3-MC(25 mg/2 ml of olive oil/kg of body weight, i.p.) was administered to pregnant rats once daily on the 18th and the 19th day of pregnancy. PB sodium (60 mg/2 ml of normal saline/kg of body weight, i.p.) was administered to pregnant rats once daily on the 18th, the 19th and the 20th day of pregnancy. The 3-MC-treated or PB-treated pregnant rats of the 21st day of pregnancy were sacrificed. Both maternal and fetal livers were immediately excised.

hemoprotein in maternal liver microsomes, the ratio was 0.40. The data obtained with 3-MC- and PB-induced hemoprotein in maternal liver microsomes are similar to those previously reported(13,14). With the hemoprotein induced in fetal liver microsomes by the maternal administration of 3-MC, the ratio of 455/430 nm was 0.42 which was about the same that observed in PB-pretreated maternal liver microsomes. In addition, the peak positions of EtCN-difference spectra of 3-MC-induced hemoprotein in fetal liver microsomes and PB-induced hemoprotein in maternal liver microsomes were the same of 429 and 454 nm. The results of EtCN as the ligand presented a strong evidence as the hemoprotein like PB-induced type cytochrome P-450 is induced in fetal liver microsomes by the maternal administration of 3-MC. Further examination was performed to characterize the hemoprotein induced in fetal liver microsomes by the use of diethylphenylphosphine as the ligand. Diethylphenylphosphine has been reported to react with the hemoprotein induced in male liver microsomes by pretreatment with PB and show characteristic difference spectra(15). Table 3 shows that the induced hemoprotein in fetal liver microsomes by the maternal administration of 3-MC also reacted with diethylphenylphosphine and showed similar difference specta. From these results, we would like to conclude that the hemoprotein induced in fetal liver microsomes by the maternal administration of 3-MC is different from that induced in maternal liver microsomes and has a property of cytochrome P-450

induced in male liver microsomes pretreated with PB. The diethylphenylphosphine-difference spectra, however, showed a small difference spectra by 1 nm between the hemoprotein induced in fetal liver microsomes by the maternal administration of 3-MC and the hemoprotein induced in maternal liver microsomes pretreated with PB. Thus, we propose here that the maternal administration of 3-MC induces hemoprotein in fetal liver microsomes which is different from cytochrome P-448 induced in maternal liver microsomes and the property is like a PB-induced cytochrome P-450. As the result of the induction of hemoprotein like PB-induced cytochrome P-450 in fetal liver microsomes by the maternal administration of 3-MC, the AM- and the HB-metabolizing activities are induced in a different manner to maternal liver microsomes. The conclusive proof of the identity of species of 3-MC-induced hemoprotein in fetal liver microsomes with PB-induced hemoprotein in maternal liver microsomes would be achieved by the use of purified cytochrome P-450 in fetal liver microsomes. We are now under the purification of cytochrome P-450 in fetal liver microsomes.

REFERENCES

1. Nebert,D.W. and Gelboin,H.V. (1969) Arch. Biochem. Biophys. 134, 76-89
2. Henderson,P.Th. (1971) Biochem. Pharmacol. 20, 1225-1232
3. Oesch,F. (1975) FEBS Lett. 53, 205-210
4. Bresnick,E. and Stevenson,J.G. (1968) Biochem. Pharmacol. 17, 1815-1822
5. Thomas,M. and Mannering,G.J. (1977) Biochem. Pharmacol. 26, 567-575
6. Cresteil,Th., Flinois,J.P., Pfister,A. and Leroux,J.P. (1979) Biochem. Pharmacol. 28, 2057-2063
7. Inoue,K., Takanaka,A., Mizokami,K., Fujimori,K., Sunouchi,M., Kasuya,Y. and Omori,Y. (1981) Toxicol. Appl. Pharmacol. 59, 540-547
8. Kato,R., Takanaka,A. and Takayanagi,M. (1970) J. Biol. Chem. 68, 395-413
9. Omura,T. and Sato,R. (1964) J. Biol. Chem. 239, 2370-2378
10. Johannessen,K.A.M. and DePierre,J.W. (1978) Anal. Biochem. 89, 725-732
11. Tangen,O., Jonsson,J. and Orrenius,S. (1973) Anal. Biochem. 54, 597-603
12. Nishibayashi,H., Omura,T. and Sato,R. (1966) Biochem. Biophys. Acta. 118, 651-654
13. Sladek,N.E. and Mannering,G.J. (1966) Biochem. Biophys. Res. Commun. 24, 668-674
14. Alvares,A.P., Schilling,G.S., Levin,N.W. and Kuntzman,R. (1976) Biochem. Biophys. Res. Commun. 29, 521-526
15. Mansuy,D., Duppel,W., Ruf,H.-H. and Ullich,V. (1974) Hoppe-Seyer's Z. Physiol. Chem. 355, 1341-1349
16. Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951) J. Biol. Chem. 193, 265-275