Biochemical Pharmacology, Vol. 30, No. 22, pp. 3095–3097, 1981.
Printed in Great Britain.

0006-2952/81/223095–03 $02.00/0

EFFECT OF CADMIUM ON HEPATIC MIXED-FUNCTION OXIDASES DURING THE EARLY DEVELOPMENT OF RATS

POSSIBLE PROTECTIVE ROLE OF METALLOTHIONEIN

PARTHASARATHI ASOKAN, RAKESH DIXIT, HASAN MUKHTAR* and COIMBATORE R. KRISHNA MURTI†

Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow 226 001, India

(*Received* 22 *December* 1980; *accepted* 15 *April* 1981)

Abstract—Hepatic metallothionein contents and activities of mixed function oxidases in control and cadmium-treated rats (1.2 mg Cd^{2+}/kg) of various age groups (7-, 14-, 21- and 90-days-old) were determined. A significantly high concentration of native metallothionein was noticed in immature rats (7- and 14-days-old). Cadmium administration induced metallothionein only in 21- and 90-day-old rats, while the basal level of native metallothionein of immature rats was not altered. Activity of certain mixed-function oxidases (MFO) such as aryl hydrocarbon hydroxylase, aminopyrine-*N*-demethylase, and benzphetamine-*N*-demethylase was inhibited significantly only in 21- and 90-day-old rats. Microsomal cadmium accumulation was significantly higher in adult as compared to immature rats. Results suggest a protective role of metallothionein against cadmium-induced inhibition of mixed-function oxidases in immature rats.

It is now well established that cadmium at acute and chronic doses induces the synthesis of metallothionein, a low molecular weight, thiol-rich protein [1–3]. The biological significance of metallothionein in detoxication of heavy metals is a subject of extensive research [4, 5]. although it has been assumed that the synthesis of metallothionein occurs only after the administration of cadmium, a very high concentration of native metallothionein in hepatic cytosols of neonates has been reported recently [6, 7]. The role of such a high concentration of metallothionein still remains unexplained.

Administration of a single dose of cadmium inhibits the activities of hepatic mixed-function oxidases in rats [8–10]. It was of interest to study whether the relatively high concentration of native metallothionein present in immature rats may have some role in influencing the cadmium-induced inhibition of mixed-function oxidases. The effect of cadmium on hepatic metallothionein levels and activities of mixed-function oxidases of rats during their early development is described in this communication.

MATERIALS AND METHODS

Animals. Female albino rats with male pups were received from the ITRC animal breeding colony. Litter size was restricted to eight with each dam, and they were allowed free access to food (Hind Lever Ltd., Bombay) and water *ad lib.*

Isolation and estimation of metallothionein. A single intraperitoneal dose of cadmium (1.2 mg Cd^{2+}/kg as $CdCl_2H_2O$) in 0.15 M NaCl was administered to male rats of different age groups (7, 14, 21 and 90 days). Control animals received the same volume of 0.15 M NaCl in an identical manner. Animals were killed after 72 hr, and livers were rapidly excised, minced and homogenized in ice-cold 0.25 M sucrose containing 10 mM Tris buffer (pH 8.2) using a Potter–Elvejhem homogenizer fitted with a Teflon pestle. This homogenate was centrifuged at 10,000 *g* for 20 min followed by further centrifugation at 105,000 *g* for 1 hr to isolate organelle-free cytosol.

A Sephadex G-75 column (2.5 × 85 cm) was equilibrated with 0.01 M Tris buffer (pH 8.2). Liver cytosol (9 ml) was applied to the column and eluted with 0.01 M Tris buffer (pH 8.2). Fractions (10 ml) were collected at a flow rate of 30 ml/hr. Void volume (V_0) was measured by eluting blue dextran 2000 with the same buffer. An adequate portion of each fraction in the range from 2.0 to 2.3 of V_e/V_0 containing cadmium was precipitated with an equal volume of Tsuchiya's reagent, and protein in metallothionein fractions was estimated by the biuret method [11].

Assay of hepatic mixed-function oxidases. Another batch of male rats of different age groups (7, 14, 21 and 90 days) were given a single intraperitoneal dose of cadmium (1.2 mg Cd^{2+}/kg as $CdCl_2H_2O$) in 0.15 M NaCl. After 72 hr, liver was removed and a 20% homogenate was prepared in cold phosphate buffer 0.1 M, pH 7.4) containing 0.15 M KCl. The crude homogenate was centrifuged at 9000 *g* for 20 min to isolate post-mitochondrial fraction for the assay of mixed-function oxidase activity.

The activities of aminopyrine and benzphetamine demethylases were assayed by measuring the formaldehyde formation [12]. Aryl hydrocarbon hydroxylase activity was assayed using

* Present address: Medical Research, Cleveland Veterans Administration Medical Center, 10701 East Boulevard, Cleveland, OH 44106, U.S.A.

† To whom reprint requests should be addressed.

benzo[*a*]pyrene as substrate by the method of Dehnen *et al.* [13]. Glutathione-*S*-transferase activity towards 1-chloro 2,4-dinitrobenzene was assayed spectrophotometrically by the method of Habig *et al.* [14]. Protein was estimated using bovine serum albumin as standard [15].

Determination of cadmium in liver microsomes of immature and mature rats. Immature (14-days-old) and adult rats (90-days-old) were treated with a single intraperitoneal injection of cadmium chloride (1.2 mg Cd^{2+}/kg) in 0.15 M NaCl. Rats were killed 6 and 72 hr after cadmium treatment. Liver homogenate prepared in 0.25 M sucrose in 0.01 M Tris buffer (pH 8.2) was centrifuged at 9000 *g* to remove cell debris, nuclei and mitochondria. Resulting post-mitochondrial fractions were subsequently centrifuged at 105,000 *g* for 1 hr to recover microsomes. Suitable portions of crude homogenate and microsomal fraction were wet digested with acid mixture for colorimetric estimation of cadmium as described in Ref. 16.

RESULTS

Effect of cadmium on hepatic metallothionein levels. The results shown in Fig. 1 represent the levels of protein in metallothionein fractions of liver cytosols of rats of different age groups exposed to a sub-acute dose of cadmium. It is evident that the immature rats (7- and 14-days-old) possessed a significantly higher concentration of native metallothionein, which was found to be decreased significantly in 21- and 90-day-old rats. Exogenous administration of cadmium, however, failed to alter the metallothionein levels in livers of immature rats. In contrast, a significant induction of metallothionein was noticed in 21- and 90-day-old rats. However, the induction of metallothionein at 90 days was higher as compared to that seen at 21 days of age.

Effect of cadmium on activities of hepatic mixed-function oxidases. The data presented in Fig. 2 show the effect of cadmium exposure on hepatic aryl hydrocarbon hydroxylase (AHH), aminopyrine-*N*-demethylase (APD), benzphetamine-*N*-demethylase (BPD) and glutathione-*S*-transferase (GST) activities during different stages of early development. Exposure to cadmium resulted in significant inhibition of APD, BDP and AHH activities only in 90-day-old rats. In contrast, no inhibition of above enzymes was seen in immature rats (7- and 14-days-old). However, in 21-day-old rats, the extent of inhibition was only marginal ($P < 0.05$). GST activity was not altered in any of the age groups studied.

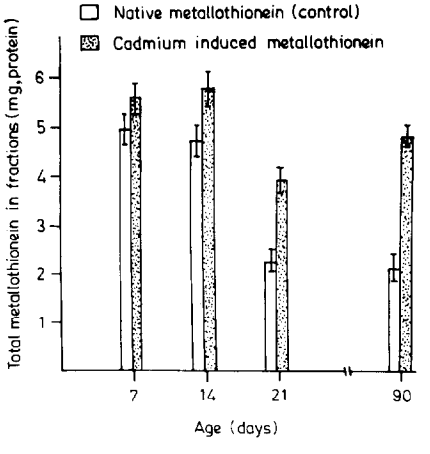

Fig. 1. Effect of cadmium administration (1.2 mg Cd^{2+}/kg as $CdCl_2H_2O$, i.p.) on the hepatic metallothionein content of developing rats. Brackets on the bar indicate standard errors. Details of the isolation and estimation of metallothionein are provided in the text.

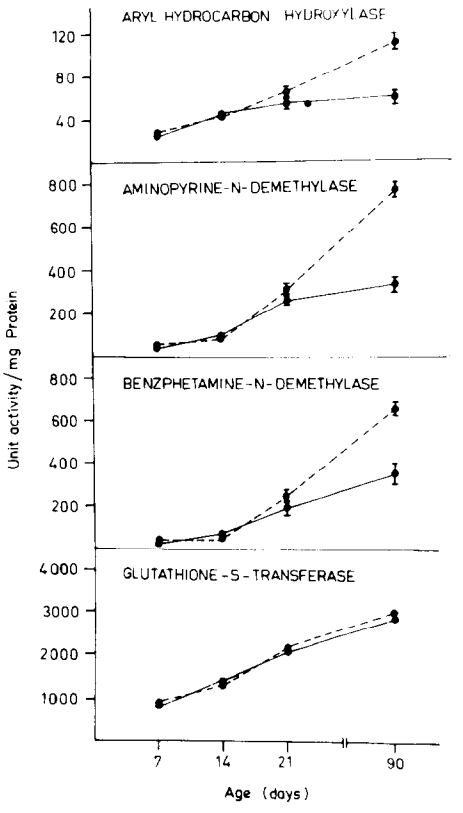

Fig. 2. Effect of cadmium administration (1.2 mg Cd^{2+}/kg as $CdCl_2H_2O$, i.p) on hepatic aryl hydrocarbon hydroxylase, aminopyrine-*N*-demethylase, benzphetamine-*N*-demethylase and glutathione-*S*-transferase activities during early development. Key: (----) control and (——) cadmium-treated (mean ± S.E.). For further details see Materials and Methods.

Table 1. Hepatic microsomal cadmium accumulation at different intervals after a single i.p. administration of cadmium chloride in adult and immature rats*

Age (days)	Cadmium (% of total accumulation)	
	6 hr	72 hr
14	2.38 ± 0.20	2.33 ± 0.13
90	3.14 ± 0.14†	3.81 ± 0.10‡

* Each value is the mean ± S.E. of four samples. P values were calculated between immature and adult rats.

† $P < 0.005$.

‡ $P < 0.001$.

Cadmium accumulation in liver microsomes of immature and adult rats. Uptake of cadmium by liver microsomes of 14- and 90-day-old rats at 6 and 72 hr following cadmium administration is shown in Table 1. Accumulation of cadmium in microsomes of 90-day-old rats was appreciably higher than that of 14-day-old rats at both 6 and 72 hr after cadmium administration.

DISCUSSION

Substantial evidence has been presented to show that administration of cadmium to adult rats leads to the synthesis of metallothionein [1–3]. It has been assumed that induction of metallothionein by cadmium is a protective mechanism by which animals defend against cadmium toxicity [17–19]. The current study reveals that induction of metallothionein by cadmium is not identical in young and adult rats. Failure of exogenously administered cadmium to trigger the synthesis of metallothionein in young rats is presumably due to the presence of a very high concentration of native metallothionein. Interestingly, other studies have also indicated the presence of a high concentration of metallothionein in hepatic cytosols of immature rats [6, 7]. Immature rats possess a significantly higher concentration of native metallothionein which might be sufficient to trap a major fraction of administered cadmium. It has been reported that most of the zinc (80 per cent) present in the native metallothionein could be replaced by cadmium at saturating levels [6]. Further, native metallothionein of immature rats has a considerable amount (30 per cent) of unoccupied binding sites [20]. In view of these observations, it is evident that cadmium could bind with the native metallothionein present in the immature rats without inducing metallothionein-like proteins.

Adult rats possess a low concentration of native metallothionein. Therefore, to stimulate the disposal of cadmium, the metal itself would have induced the metallothionein levels. Although the native metallothionein content was apparently the same at 21 and 90 days of age, as judged from the protein content of metallothionein fractions, the native metallothionein content would be expected to be higher at 21 days than that at 90 days because in our earlier studies it has been demonstrated that the concentration of sulfhydryl groups is higher at 21 days compared to that at 90 days [21]. It is known that in the metallothionein fractions approximately 70 per cent of the sulfhydryl groups originate from metallothionein-like proteins [22]. A significant amount of metallothionein in rats at 21 days compared to that at 90 days would lead to considerable binding of administered cadmium, resulting in lower induction of metallothionein and thereby in lower inhibition of MFO enzymes.

It was also interesting to note that young rats were insensitive towards inhibitory effects of cadmium on hepatic MFO enzyme activities. It is now well established that cadmium is a strong inhibitor of MFO enzymes in adult rats [8–10]. However, the inability of cadmium to exert inhibitory effects on MFO enzyme activities in immature rats is possibly due to a protective role provided by native metallothionein.

Native metallothionein would have also reduced accumulation of cadmium at the site of microsomal MFO metabolism. In contrast, a sufficient fraction of administered cadmium would have reached microsomes before the onset of metallothionein synthesis to inhibit microsomal MFO enzyme activities in adult rats.

It was significant to observe that accumulation of cadmium was appreciably higher in liver microsomes of adult rats following exposure to cadmium as compared to immature rats. This indicates that the higher concentration of metallothionein in immature rats reduced microsomal uptake of cadmium and thus native metallothionein plays an important role in providing protection against cadmium-induced inhibition of MFO enzyme activities. Studies by Teare *et al.* [23] have also shown that the degree of inhibition of MFO enzymes by cadmium depends on the amount of metal accumulated in microsomes.

Our study suggests a protective role of native metallothionein against cadmium-induced biochemical alterations, such as inhibition of activities of MFO enzymes in immature rats.

Acknowledgements—The authors are thankful to Dr. P. K. Seth for supporting the present work. Thanks are also due to Mr. J. S. Martolia for his technical assistance and to Mrs. Rosemary Davis for typing.

REFERENCES

1. M. Piscator, *Nord. hyg. Tidskr.* (in Swedish) **45**, 76 (1964).
2. D. R. Winge, R. Premakumar and K. V. Rajagopalan, *Archs Biochem. Biophys.* **170**, 242 (1975).
3. Z. A. Shaikh and O. J. Lucis, *Fedn Proc.* **29**, 298 (1970).
4. M. G. Cherian and R. A. Goyer, *Life Sci.* **23**, 1 (1978).
5. M. Nordberg, *Envir. Res.* **15**, 381 (1978).
6. J. U. Bell, *Toxic. appl. Pharmac.* **50**, 101 (1979).
7. H. Ohtake, K. Hasegawa and M. Koga, *Biochem. J.* **174**, 999 (1978).
8. W. M. Hadley, T. S. Miya and W. F. Bousquet, *Toxic. appl. Pharmac.* **28**, 284 (1974).
9. T. Yoshida, Y. Ito and Y. Suzuki, *Bull. envir. Contam. Toxic.* **15**, 402 (1976).
10. R. R. Dalvi and T. J. Robbins, *J. envir. Path.* **1**, 601 (1978).
11. M. Piscator and B. Pettersson, in *Clinical Chemistry and Chemical Toxicology of Metals* (Ed. S. S. Brown), p. 143. Elsevier, North Holland (1977).
12. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
13. W. Dehnen, R. Tomingas and J. Ross, *Analyt. Biochem.* **53**, 373 (1973).
14. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. B. E. Saltzman, *Analyt. Chem.* **25**, 493 (1953).
17. C. Terharr, E. Vis, R. Roudabush and D. Fassett, *Toxic. appl. Pharmac.* **7**, 500 (1968).
18. G. Gabbiani, D. Baic and C. Deziel, *Can. J. Physiol. Pharmac.* **45**, 443 (1966).
19. A. P. Leber and T. S. Miya, *Toxic. appl. Pharmac.* **37**, 403 (1976).
20. J. U. Bell, *Toxic. appl. Pharmac.* **54**, 148 (1980).
21. P. Asokan and S. K. Tandon, *Envir. Res.* **24**, 201 (1981).
22. M. Nagahashi, O. Wada, T. Ono, N. Yamaguchi and K. Takeo, *Ind. Health* **12**, 31 (1974).
23. F. W. Teare, P. R. Read, R. B. Pyttel and P. A. Jasansky, *Archs envir. Hlth* **33**, 53 (1978).