Arch. Toxicol. 40, 269–277 (1978)

Archives of
TOXICOLOGY

Effect of Acute and Chronic Cadmium Treatment on Hepatic Drug Metabolism in Male Rats

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Abstract. The effect of acute and chronic cadmium administration on hepatic drug metabolism was investigated in the male rat. 3 days after the acute administration of cadmium by either the intraperitoneal (0.84 mg Cd/kg) or the oral (> 80 mg Cd/kg) route, there was a significant potentiation in duration of hexobarbital hypnosis and inhibition of hepatic microsomal metabolism of hexobarbital and aniline. Administration of cadmium in the drinking water at levels of 100 or 200 ppm Cd for periods of 2–12 weeks or at levels of 5 or 20 ppm Cd for 50 weeks did not produce alterations in either drug response or hepatic drug metabolism. Significant levels of metallothionein, a cadmium binding protein, found in the liver of the rats receiving cadmium chronically may offer an explanation for the observed differences in drug metabolism between the acute and chronic administration of cadmium. In additional studies, pretreatment of the rats with subthreshold doses of cadmium (0.21 or 0.42 mg Cd/kg) intraperitoneally produced a tolerance to the alterations in drug metabolism induced by the previous cadmium dose (0.84 mg Cd/kg, i.p.). However, chronic cadmium treatment (5 or 20 ppm Cd for 50 weeks) did not impart any such tolerance to subsequently administered Cd (0.84 mg/kg) by the intraperitoneal route. The hepatic levels of metallothionein induced by the chronic cadmium treatment were only 30–60% of those induced by the subthreshold cadmium and thus may not have bound enough of the large challenge cadmium dose to produce the tolerance phenomenon.

Key words: Cadmium – Drug Metabolism – Metallothionein – Rat.

Introduction

Cadmium is highly toxic to biological systems, inducing a wide variety of adverse manifestations in both laboratory animals and man (reviewed by Flick et al., 1971; Louria et al., 1972; Friberg et al., 1974). However, an apparent difference in the toxicity of cadmium is observed when the metal is administered on an acute basis as

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0340-5761/78/0040/0269/$ 01.80

compared to chronic administration. Differences have been reported for toxic manifestations such as testicular necrosis (Nordberg, 1971) and hyperglycemia (Ghafghazi and Mennear, 1973) induced by acute cadmium administration, but these toxicities were no longer observed after chronic administration of the metal. Merali et al. (1975) found a stimulation in the activity of four gluconeogenic enzymes in rat liver after chronic cadmium administration but not after acute administration of the metal.

A partial explanation for these observed differences is related to the observation that cadmium in low doses stimulates the formation of metallothionein, a cadmium-binding protein (Webb, 1972; Squibb and Cousins, 1974), which performs a detoxification function, sequestering the subsequently administered cadmium and rendering it inert (Nordberg, 1971; Leber and Miya, 1976; Probst et al., 1977b).

In many previous studies, we have reported that the acute administration of Cd in intraperitoneal doses greater than 0.84 mg Cd/kg produces a potent inhibition of hepatic drug metabolism in male rats (Hadley et al., 1974; Johnston et al., 1975; Roberts et al., 1976; Pence et al., 1977). In contrast to these results, Wagstaff (1973) has reported that Cd given in the diet (100–5000 ppm for 15 days) produces a stimulation in hepatic drug metabolism in female rats. Becking (1976) found that administration of Cd in drinking water (2–200 ppm) for period of 60–180 day to male rats did not alter hepatic drug metabolism.

The objective of this study was to assess comparatively the effects of acute and chronic administration of cadmium on hepatic drug metabolism in the male rat.

Methods

Animals. Male, Sprague-Dawley derived rats (Laboratory Supply Co., Indianapolis, Ind.) were used throughout these studies. In the acute studies, the animals were housed in community cages for 10–14 days and allowed free access to food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and tap water. In the chronic studies, the animals were housed either individually or in community cages as indicated.

Chemicals. Cadmium acetate (Fisher Scientific Co., Fair Lawn, N.J.) was dissolved in double-distilled water prior to administration by the intraperitoneal or oral routes or in the drinking water. Controls received sodium acetate in such a dose or concentration as to receive acetate ion equimolar to the acetate concentration in the cadmium solutions.

Pharmacological Response. The duration of hexobarbital hypnosis was measured as the time elapsing from the loss of the righting reflex until the animal could successfully right itself from the supine position twice within 30 s.

Drug Metabolism. Hepatic drug metabolism of various substrates was measured using the 105,000 $\times$ g_{max} microsomal pellet as previously described (Hadley et al., 1974; Pence et al., 1977). Hexobarbital was determined by the method of Brodie et al. (1953) and aniline was determined by the method of Imai et al. (1966).

Metallothionein Concentrations. Hepatic metallothionein concentrations in the 105,000 $\times$ g_{max} supernatant were measured by the method described by Probst et al. (1977a, b) and Yau and Mennear (1977). This method estimates the total cadmium binding capacity of the metallothionein.

Statistical Analysis. The data were analyzed statistically by use of analysis of variance (ANOVA) followed by application of Newman-Keuls test where appropriate (Anderson and McLean, 1974).

Results

Effect of Acute Cadmium Administration on Hepatic Drug Metabolism

Typical results showing the influence of an acute intraperitoneal dose of cadmium (0.84 mg/kg, i.p.) on drug response and hepatic drug metabolism in the male rat are shown in Table 1. The duration of hexobarbital hypnosis was significantly ($p < 0.01$) prolonged and the rate of hepatic drug metabolism using either hexobarbital or

Table 1. Effect of acute intraperitoneal administration of cadmium on hexobarbital hypnosis and hepatic drug metabolism im male rats[a]

Treatment	Duration of hypnosis (min ± SE)	Hepatic microsomal metabolism	
		Hexobarbital oxidase $\left(\frac{\mu\text{mole metabolized}}{\text{mg protein} \cdot \text{h}}\right)$	Aniline oxidase $\left(\frac{\text{nmole product}}{\text{mg protein} \cdot 20\text{ min}}\right)$
Sodium acetate (1.23 mg/kg)	23.6 ± 1.4 (6)	1.00 ± 0.04 (7)	32.7 ± 1.1
Cadmium acetate (0.84 mg Cd/kg)	59.2 ± 6.6[b] (6)	0.27 ± 0.10[b] (8)	12.2 ± 0.7[b]

[a] Adult, male rats (180–220 g) received cadmium acetate (0.84 mg Cd/kg, i.p.) and the duration of hexobarbital (100 mg/kg, i.p.) hypnosis or rate of hepatic microsomal drug metabolism were measured 72 h later. Controls received sodium acetate (1.23 mg/kg, i.p.). Data are expressed as mean ± SE. Number of animals per group are shown in parentheses

[b] Significantly different from control ($p < 0.01$)

Table 2. Effect of acute oral cadmium administration on hexobarbital hypnosis and microsomal drug metabolism[a]

Treatment (mg Cd/kg, p.o.)	Duration of hexobarbital hypnosis (min ± SE)	Hepatic drug metabolism	
		Hexobarbital oxidase $\left(\frac{\mu\text{mole metabolized}}{\text{mg protein} \cdot \text{h}}\right)$	Aniline oxidase $\left(\frac{\text{nmole product}}{\text{mg protein} \cdot 20\text{ min}}\right)$
0	14.5 ± 0.8 (6)	0.98 ± 0.05 (4)	44.6 ± 3.3
40	20.7 ± 4.1 (4)	0.96 ± 0.07 (6)	45.1 ± 1.7
80	26.1 ± 2.2[b] (8)	0.54 ± 0.06[b] (7)	32.4 ± 2.2[b]
120	34.2 ± 3.8[b] (8)	0.33 ± 0.02[b] (5)	25.6 ± 2.6[b]

[a] Adult, male rats (180–220 g) received cadmium orally in the doses indicated and the duration of hexobarbital hypnosis (100 mg/kg, i.p.) or rate of hepatic microsomal drug metabolism were measured 72 h later. Controls received sodium acetate. Data are expressed as mean ± SE. Number of animals per group are shown in parentheses

[b] Significantly different from control ($p < 0.01$)

aniline as the substrate was significantly ($p < 0.01$) inhibited 3 days after cadmium administration. The concentration of cadmium in liver tissue at this time was 6 μg/g wet tissue as determined by atomic absorption spectrometry.

An experiment was also conducted to examine the effect of an acute oral dose of cadmium on drug response and hepatic drug metabolism. The results presented in Table 2 indicate that at doses of 80 or 120 mg Cd/kg duration of hexobarbital hypnosis was prolonged significantly ($p < 0.01$) and hepatic microsomal metabolism of hexobarbital and aniline was inhibited significantly ($p < 0.01$). In separate studies (data not shown), cadmium was administered in doses ranging from 10–300 mg Cd/kg and all doses of cadmium at 80 mg Cd/kg or greater were effective in prolonging the duration of hexobarbital hypnosis. The oral LD50 of cadmium was 175 mg Cd/kg.

Effect of Chronic Cadmium Treatment on Hepatic Drug Metabolism

The effect of chronic cadmium administration on these drug action parameters is shown in Tables 3 and 4. In the first experiment, these rats received cadmium in the drinking water at concentrations of 100 or 200 ppm for varying intervals of time between 2 and 12 weeks.

As can be seen from data presented in Table 3, the chronic cadmium treatment did not induce any alterations in duration of hexobarbital hypnosis or hepatic microsomal metabolism. Since these animals were housed individually, the amount of drinking water consumed was measured on a daily basis and the total cadmium intake for the treatment period was recorded. Thus, these animals consumed a total amount of 43.1–233.5 mg of cadmium. Calculated on a per animal basis these Cd doses are greater than the acute dose of cadmium administered in the previous

Table 3. Effect of chronic oral cadmium administration on duration of hexobarbital hypnosis and hepatic microsomal hepatic drug metabolism in male rats[a]

Treatment Cd (ppm)	Duration of treatment (weeks)	Total Cd consumed (mg)	Duration of hexobarbital hypnosis (min ± SE)	Hepatic hexobarbital $\left(\frac{\mu\text{mole metabolized}}{\text{mg protein} \cdot \text{h}}\right)$
0	12	0	19.2 ± 1.1 (6)	1.24 ± 0.12 (6)
100	2	43.1 ± 2.3	17.9 ± 1.8 (8)	1.43 ± 0.11 (8)
100	4	89.6 ± 2.5	16.5 ± 0.9 (7)	1.13 ± 0.13 (6)
100	12	233.5 ± 19.8	16.4 ± 1.5 (6)	1.04 ± 0.08 (6)
200	2	61.2 ± 3.3	21.7 ± 3.1 (7)	1.10 ± 0.10 (6)
200	4	154.9 ± 7.6	18.2 ± 0.8 (7)	1.12 ± 0.07 (6)

[a] Adult, male rats were housed individually and cadmium was administered in the drinking water in the concentrations and for the time intervals as designated controls received sodium acetate. Hypnosis was induced by hexobarbital (100 mg/kg, i.p.). Hexobarbital metabolism was measured in hepatic microsomes as described

Table 4. Effect of chronic cadmium administration on duration of hexobarbital hypnosis and microsomal hepatic drug metabolism[a]

Treatment	Duration of hypnosis (min ± SE)	Hepatic microsomal drug metabolism	
		Hexobarbital $\left(\frac{\mu\text{mole metabolized}}{\text{mg protein}}{\text{h}}\right)$	Aniline $\left(\frac{\text{nmole product}}{\text{mg protein}}{20\text{ min}}\right)$
0	40.8 ± 2.3	123.0 ± 10.3	35.3 ± 0.9
5	29.8 ± 3.1	138.3 ± 9.9	35.7 ± 1.2
20	35.5 ± 3.7	160.2 ± 12.4	36.6 ± 0.9

[a] Adult male rats (430–575 g) received cadmium acetate (5 or 20 ppm Cd) for 50 weeks in the drinking water. Controls received sodium acetate. Hypnosis was induced by hexobarbital (150 mg/kg, i.p.) and rate of metabolism was measured using hepatic microsomes as described. Each value represents the mean ± SE for eight animals

experiment (Table 2). Thus, chronic consumption of low cadmium doses on a daily basis does not influence drug action in the male rat.

In another study rats received cadmium in the drinking water at concentrations of 5 or 20 ppm for a duration of 50 weeks. The data presented in Table 4 indicate that this treatment did not alter either hexobarbital hypnosis or hepatic drug metabolism.These animals were housed in community cages of six animals each and, therefore, no estimate could be made of total cadmium intake.

Effect of Acute and Chronic Cadmium Treatment on Cadmium-Induced Tolerance to Cadmium Potentiation of Drug Response

Previous studies have reported that certain cadmium-induced toxicities (e.g., testicular necrosis and acute lethality) may be prevented by pretreating the animals with cadmium in doses which are too low to induce necrotic damage or induce death (Nordberg, 1971; Leber and Miya, 1976; Probst et al., 1977b). This appears to be the case in regard to the cadmium-induced alterations in drug action (Roberts et al., 1976; Yoshida et al., 1977). Pretreatment of male rats with a subthreshold dose of cadmium (0.21 mg Cd/kg, i.p.; Schnell et al., 1974) 3 days prior to administering a challenge cadmium dose (0.84 mg Cd/kg, i.p.) produces a tolerance phenomenon in which the cadmium induced alterations in drug action are no longer observed (Fig. 1).

We examined this same phenomenon in rats receiving cadmium (5 or 20 ppm) in the drinking water for 50 weeks. As can be observed from the data in Figure 2, there was no tolerance phenomenon exhibited to the prolongation of hexobarbital hypnosis in rats receiving cadmium orally for prolonged periods of time.

The underlying basis for this cadmium induced tolerance phenomenon may be related to elevated levels of hepatic metallothionein, a cadmium-binding protein, synthesized in response to cadmium exposure. The data in Figure 3 depict the levels

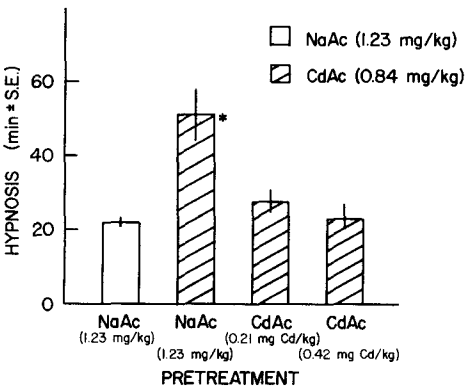

Fig. 1. Influence of cadmium pretreatment on cadmium-induced potentiation of hexobarbital hypnosis. Adult, male rats (220–270 g) were pretreated with cadmium acetate (0.21 or 0.42 mg Cd/kg, i.p.). 3 days later these rats received another cadmium dose (0.84 mg/kg, i.p.). Control animals were treated with sodium acetate (1.23 mg/kg, i.p.). 3 days after this second injection the duration of hypnosis induced by hexobarbital (100 mg/kg, i.p.) was measured. Data are expressed as mean ± SE. Each group contained eight to nine rats

* Significantly different from control ($p < 0.05$)

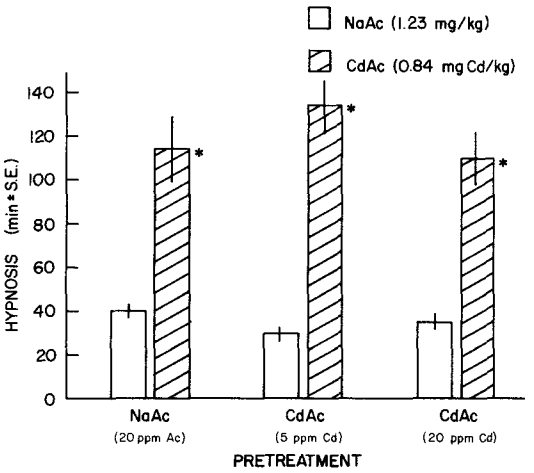

Fig. 2. Influence of acute cadmium on hexobarbital hypnosis in male rats chronically treated with cadmium. Adult male rats (475–625 g) received cadmium acetate (5 or 20 ppm Cd) for 50 weeks in the drinking water. Controls received sodium acetate. Each of these three groups was further divided into two groups which received an injection of either sodium acetate (1.23 mg/kg, i.p.) or cadmium acetate (0.84 mg Cd/kg, i.p.). 3 days later each animal received hexobarbital (150 mg/kg, i.p.) and duration of hypnosis was measured. Each bar represents the mean ± SE of eight animals

* Significantly different from the respective control ($p < 0.01$)

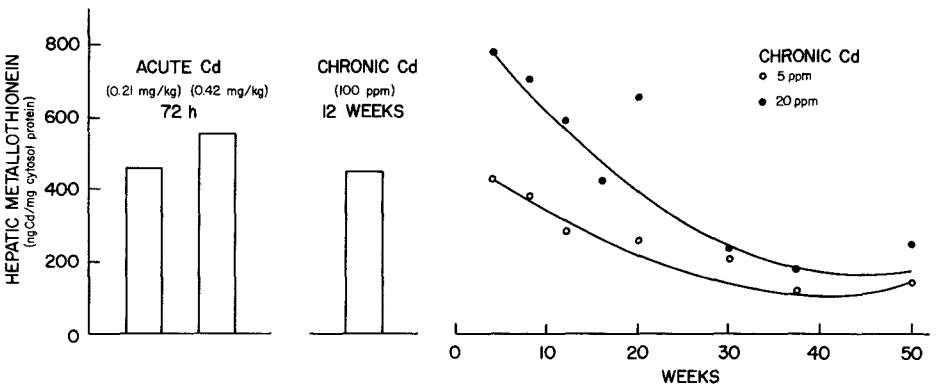

Fig. 3. Total hepatic metallothionein levels in male rats following acute or chronic treatment with cadmium. Adult, male rats received cadmium either intraperitoneally or orally in the doses and times as designated. Total hepatic metallothionein were determined as described in Methods

of total hepatic metallothionein in male rats receiving varying exposures to cadmium. In Figure 3A are hepatic levels of metallothionein measured 72 h after the rats received 0.21 or 0.42 mg Cd/kg by the intraperitoneal route. In Figure 3B are levels of metallothionein in livers of rats receiving cadmium in drinking water (100 ppm) for 12 weeks, and these levels were approximately the same as those induced by the acute cadmium administration. In Figure 3C are the levels of total hepatic metallothionein measured in rats at intervals of 4–50 weeks receiving 5–20 ppm Cd in drinking water. At the period of 50 weeks the final metallothionein levels were only 30–60% of those rats exhibiting the tolerance phenomonon.

Discussion

Following the acute administration of cadmium there is a prolongation of hexobarbital hypnosis and an inhibition of hepatic drug metabolism regardless of the route of administration. For both the intraperitoneal route and oral route of administration, there is a clear threshold dose of cadmium required to alter drug action, and interestingly, the oral intraperitoneal dose ratio is about 100/1. This correlates quite well with the observation that only 1–2% of an oral dose of cadmium is absorbed (Decker et al., 1957). Kotsonis and Klaassen (1977) have reported that a similar relationship exists where comparing LD50 values in male rats from oral (225 mg Cd/kg) and intraperitoneal (3.35 mg Cd/kg) routes.

Our finding that oral cadmium potentiates hexobarbital hypnosis and inhibits hepatic microsomal drug metabolism contrasts to the data reported by Kotsonis and Klaassen (1977). These investigators administered cadmium chloride orally in doses ranging from 0–150 mg Cd/kg but found no alteration in microsomal cytochrome P-450 content, or aniline hydroxylase activity at 2 or 14 days after cadmium, but did find a slight decrease in hexobarbital oxidase at 2 days but not 14 days after Cd. At present we have no explanation for these differences in observations.

Following the chronic administration of cadmium, no observable changes in drug action parameters were found even though the total cadmium dose was much greater than the acute oral dose required for producing such alterations in drug action. Although the importance of metallothionein in the pathogenesis of Cd toxicity is not understood, the underlying basis for this lack of toxic effect may be a reflection of the binding of cadmium to metallothionein. In this regard, Leber and Miya (1976) and Probst et al. (1977b) have demonstrated that prior administration of non-lethal doses of Cd increases the LD50 to cadmium in mice; and, furthermore, they have demonstrated that levels of hepatic metallothionein are directly proportional to the pretreatment dose of cadmium and are positively correlated with the observed increase in cadmium LD50 values. In our experiments we have found substantial levels of hepatic metallothionein in rats receiving cadmium in the drinking water. However, in the rats receiving cadmium for 50 weeks there was noted decline in metallothionein concentration over the period (4–50 weeks) of measurement. The data reported by Squibb et al. (1976) raise the question as to whether or not new cadmium was actually reaching the liver in view of the fact that cadmium also stimulates the formation of metallothionein within the intestinal tissue. The

intestinal metallothionein may have prevented any further cadmium from being absorbed from the relatively low levels (5 and 20 ppm) of metal to which the rats were exposed. Thus, the decline in hepatic metallothionein may represent the normal decline of cadmium and this protein without exposure to new free metal to stimulate its synthesis. Earlier studies by Cotzias et al. (1961) and Moore et al. (1963) indicate that cadmium has a rather long half-life (50–250 days) in the rodent. If metallothionein behaves as the primary sequestering agent for cadmium in the liver (Webb, 1972), then the half-life of metallothionein should approximate the half-life for cadmium or there would have to be a continual resynthesis, and rather rapid turnover, of new metallothionein. Probst et al. (1977a) have shown that effective hepatic metallothionein synthesis was terminated 48 h after metal exposure.

Chronic administration of cadmium did not alter drug response or metabolism and did not trigger those processes responsible for the development of the tolerance phenomenon reported after administration of low doses of Cd (Roberts et al., 1976; Leber and Miya, 1976; Probst et al., 1977b; and Yau and Mennear, 1977). The reason for this failure is unknown. However, one possible explanation may be that the amount of protein binding sites available to sequester the toxic cadmium dose were decreased. This is supported by the data in Figure 3 in which the concentration of metallothionein is decreased 30–60% (Fig. 3C) compared to the levels generated by the acute administration of the metal (Fig. 3A). In addition, the degree of saturation of the metallothionein in vivo by cadmium may also differ. After the acute administration of cadmium only 5–10% of the binding sites are occupied by the metal, whereas in the chronic situation approximately 26–95% of the metal binding sites are occupied depending upon the cadmium dose (unpublished observations).

In conclusion, these data indicate that cadmium administered acutely inhibits hepatic drug metabolism and alters drug response in the male rat. Administration of cadmium by the oral route in a chronic manner does not influence these drug action parameters.

Acknowledgements. The authors thank Timothy D. Prosser for his expert technical assistance. This work was supported by NIEHS Training Grants (ES-00921 and ES-01123), NIEHS Environmental Toxicology Training Grant (ES-00071), and NIH National Service Award Training Grant (1-T32-GM-07095).

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Received January 20, 1978