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CADMIUM POTENTIATION OF DRUG RESPONSE—ROLE OF THE LIVER*

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Abstract Three days after a single cadmium dose (2 mg/kg), the duration of response to subsequently administered hexobarbital or zoxazolamine was potentiated. Duration of sleep was prolonged (225 per cent) in cadmium-treated rats, but the awakening plasma hexobarbital levels were similar in both the cadmium-treated animals and saline-treated controls. Moreover, cadmium-treated animals exhibited significantly higher plasma hexobarbital levels when sacrificed prior to awakening at a time corresponding to the mean duration of sleep in control rats, thus suggesting that the cadmium effect may be mediated by a decline in the plasma disappearance of hexobarbital. Duration of hexobarbital sleep was not changed in cadmium-treated hepatectomized rats compared to hepatectomized control animals, indicating the necessity of an intact liver for the cadmium effect to be elicited. In addition, pretreatment with cadmium significantly inhibited metabolism of hexobarbital *in vitro* in the isolated perfused rat liver (65 per cent). After the cadmium treatment, zoxazolamine-induced paralysis was prolonged (185 per cent), but the plasma drug levels measured upon recovery were significantly lower. Significantly higher plasma levels of zoxazolamine were found in cadmium-treated rats when they were sacrificed prior to recovery at a time corresponding to the mean duration of paralysis in control rats. These results thus infer that the cadmium interaction with zoxazolamine may involve both an alteration in drug disposition as well as a change in tissue responsiveness.

For over a century, cadmium (Cd) compounds have been known to be toxic to man, and the symptomology accompanying both acute and chronic intoxication has been described [1–3]. In the past, cadmium was largely recognized as an industrial hazard, but recent attention has been directed toward the role of trace levels of cadmium as an etiological factor in various chronic pathological conditions such as arteriosclerosis, hypertension, testicular tumors, renal dysfunction, emphysema, growth inhibition, cancer and chronic diseases of old age [4, 5].

Cadmium is widely spread throughout the biosphere and has currently become recognized as a toxicologically important environmental contaminant. In the United States, the daily human intake of this metal has been reported to be 200–500 μg [6]. Because cadmium is slowly excreted, the body burden accumulates progressively with age [7, 8]. The "Standard American Man" is estimated to have a total body burden of approximately 30 mg cadmium, with the major proportion being found in the kidney, pancreas and liver [9, 10]. Tipton and Cook [11] have reported cadmium levels of 30–40 ppm in the kidney and 3–4 ppm in the liver of human adults.

After cadmium exposure, various pathological changes have been reported to occur within the liver such as focal necrosis, fibrosis, fatty infiltration, cirrhosis and inflammation [12–14]. Within the hepatocyte *per se*, both the microsomal and mitochondrial fractions accumulate cadmium rapidly [15, 16]. After oral cadmium treatment (160 ppm Cd for 200 days) in the rabbit, Stowe *et al.* [17], utilizing electron microscopy, reported the most seriously affected organelle was the endoplasmic reticulum. There was a marked increase in smooth endoplasmic reticulum, especially in the cell periphery, and there was also prominent dilatation of the rough endoplasmic reticulum.

Recent studies have reported that the duration of drug response is altered after the administration of cadmium salts [18–21]. Because cadmium is known to accumulate and produce toxic changes in the liver and since the liver is the major location of drug-metabolizing enzymes, the present study was undertaken to investigate the role of the liver in the cadmium-induced alteration of drug response.

METHODS

Animals. Male, Sprague–Dawley-derived rats, weighing 280–320 g, were obtained from Laboratory Supply Co., Indianapolis, Ind. The animals were housed in community cages (six rats/cage) in an air-conditioned room maintained at 22–23° under a 14–10 light–dark cycle (L: 0600–2000) for 7–10 days prior to the experiment. Free access to food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and water was allowed. Cadmium acetate (Mallinckrodt Chemical Co., St. Louis, Mo.) was dissolved in saline and administered intraperitoneally at a dose of 2 mg/kg (840 μg/kg of cadmium ion) 72 hr prior to the experiment. Dose–response studies with Cd reveal that this dose is the minimal effective dose required to potentiate drug response. Time–course studies indicate that the maximal

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Table 1. Influence of cadmium on drug response and plasma levels *in vivo* of hexobarbital and zoxazolamine

Drug treatment	Control	Cadmium*	Paired-cadmium controls†
Hexobarbital Na (100 mg/kg)	Sleep times (min)		Time of sacrifice (min)
	22·2 ± 1·6	56·7 ± 8·3‡	22·2
	Awakening plasma levels (μg/ml)		Plasma levels (μg/ml)
	54·5 ± 2·8	55·8 ± 2·4	71·6 ± 2·2‡
Zoxazolamine HCl (70 mg/kg)	Paralysis times (min)		Time of sacrifice (min)
	164 ± 9·0	304 ± 2·6‡	164
	Recovery plasma levels (μg/ml)		Plasma levels (μg/ml)
	21·7 ± 0·4	18·7 ± 0·8‡	28·1 ± 10·6‡

* Male, albino rats (280–320 g) received either saline (1 ml/kg, i.p.) or cadmium acetate (2 mg/kg, i.p.). Three days later, the duration of pharmacological response produced either by hexobarbital Na (100 mg/kg, i.p.) or by zoxazolamine HCl (70 mg/kg, i.p.) was determined. Immediately upon termination of the pharmacological response, the animals were sacrificed and plasma samples taken for subsequent analysis for the respective drug. Each value represents a mean ± S. E. of five to six rats.

† These cadmium-treated (2 mg/kg, i.p.; 72 hr) rats were sacrificed at a time corresponding to the average duration of response recorded for the respective control groups; i.e. the hexobarbital-treated group was sacrificed at 22·2 min; the zoxazolamine-treated rats at 164 min. Plasma samples were obtained for drug analysis.

‡ Statistically different ($P = 0·05$; Student's *t*-test). All statistical comparisons were made with the control group.

potentiation of drug response occurs from day 2 to day 5 after cadmium treatment [20].

Drug response. Hexobarbital was dissolved in physiological saline and zoxazolamine in HCl as described by Conney *et al.* [22]. The duration of response to each agent was defined as the time between loss and recovery of the righting reflex. For hexobarbital, this interval is referred to as sleep time; for zoxazolamine, paralysis time.

Plasma drug levels. Plasma hexobarbital levels were analyzed by the method of Brodie *et al.* [23]; levels of zoxazolamine, by the method of Burns *et al.* [24].

Partial hepatectomy. Partial hepatectomies (65–75 per cent) were performed according to the method of Higgins and Anderson [25]. Sham-operated animals were anesthetized with ether, the peritoneum was opened, the liver exteriorized, and the wound closed as in the hepatectomized animals. All surgical procedures were performed on the same day. After surgery, the animals were housed individually and maintained on 15% dextrose solution in addition to their normal diet.

Isolated liver perfusion. The metabolism of hexobarbital and codeine (conversion to morphine) was studied using the isolated liver perfusion technique. Perfusions were carried out at 37° in an apparatus (Metro Scientific, Inc., Long Island, N.Y.) described by Burton *et al.* [26], using a medium consisting of freshly prepared, aerated (95% O_2–5% CO_2) Krebs–Ringer bicarbonate solution (pH 7·4) containing 0·05% glucose. After the perfusion rate was stabilized at 2 ml/g/min, the appropriate drug was added to the perfusion medium (total volume, 100 ml) and the livers were perfused for a period of not more than 1 hr. At designated intervals, aliquots (5 ml) of the perfusion medium were taken to determine the concentration of either hexobarbital [23] or morphine [27].

Statistical analysis. The data were analyzed for significance of difference by Student's *t*-test.

RESULTS

Influence of cadmium on drug response and drug plasma levels in vivo. We have previously reported that

Table 2. Influence of cadmium on hexobarbital-induced sleep in hepatectomized rats*

Treatment group	Sleep time (min ± S. E.)	Liver/body ratios† Day 11
Sham-operated-saline	16·8 + 1·1	0·04 ± 0·001
Sham-operated-cadmium	24·5 ± 1·6	0·04 ± 0·002
Hepatectomy-saline	68·5 ± 8·1	0·04 ± 0·001
Hepatectomy-cadmium	67·2 ± 9·3‡	0·04 ± 0·002

* Cadmium acetate (2 mg/kg, i.p.) or saline (1 ml/kg, i.p.) was administered to male rats (280–320 g) 24 hr prior to partial hepatectomy or sham-operation. Duration of sleep induced by hexobarbital Na (75 mg/kg, i.p.) was determined 48 hr after surgical manipulation and 72 hr after cadmium administration. Each value represents the mean ± S. E. for six animals.

† All animals were allowed to recover from sleep and were maintained until day 11 after surgery. Animals were then sacrificed and liver and body weights determined. After hepatectomy, Fouts *et al.* [28] have shown that liver mass completely regenerated by day 10.

‡ Not statistically different (Student's *t*-test) compared to hepatectomy-saline group.

Table 3. Influence of cadmium pretreatment on drug metabolism by the isolated perfused rat liver*

Treatment	Substrate metabolized: Hexobarbital (μmoles hexobarbital metabolized/g liver/hr)	Substrate metabolized: Codeine (μmoles morphine formed/g liver/hr)
Control	5·04 ± 0·37	2·20 ± 0·13
Cadmium	3·36 ± 0·55†	1·20 ± 0·28†

* Male rats (280–320 g) received either saline (1 ml/kg, i.p.) or cadmium acetate (2 mg/kg, i.p.). Three days later the animals were sacrificed and the livers were perfused for a period of 1 hr as described in Methods. Each value represents the mean ± S. E. for five to seven animals.

† Statistically different ($P < 0·05$, Student's t-test) from the respective control.

cadmium pretreatment may potentiate drug response [18–20]. This experiment was conducted to study the influence of cadmium treatment on the plasma levels *in vivo* of hexobarbital and zoxazolamine. The basic experimental design was similar for each drug. In each experiment, one group of cadmium-treated rats (2 mg/kg, i.p.; 72 hr) and a saline control group were treated with the respective drug, and the duration of pharmacological response (hexobarbital-sleep time; zoxazolamine-paralysis time) was determined. Immediately upon termination of the pharmacological response, animals were sacrificed and the plasma concentration of the respective drug was determined. Another group of cadmium-treated rats was administered the respective drug, then sacrificed while still sleeping (or paralyzed) at a time interval representing the mean duration of response observed in the control group, and the plasma was analyzed for the respective drug at this critical time interval (paired-cadmium control) (Table 1).

Although cadmium treatment significantly prolonged the hexobarbital-sleep time (255 per cent), it did not influence the awakening plasma levels of this drug, suggesting that central nervous system sensitivity to hexobarbital was not altered by cadmium. Plasma levels of hexobarbital in the cadmium-treated rats sacrificed at the mean duration of sleep observed for the control rats were significantly increased compared to the control levels of hexobarbital at this critical time interval. These data suggest that cadmium interferes with the plasma decline rate for hexobarbital and thus prolongs hexobarbital-sleep time.

Cadmium also significantly prolonged the duration of zoxazolamine-induced paralysis time (185 per cent). Plasma levels of the drug determined upon recovery from paralysis are higher (14 per cent) in the control rats than in the cadmium-treated animals. This difference in drug level, although small in magnitude, suggests a possible change in the tissue sensitivity to zoxazolamine. The zoxazolamine levels in the plasma of cadmium-treated rats which were sacrificed at the mean paralysis time of the controls were significantly higher (129 per cent) than in the corresponding control rats at this critical time, indicating that cadmium also interferes with the plasma decline of zoxazolamine. These data suggest that cadmium may potentiate zoxazolamine-induced paralysis by two mechanisms, i.e. (1) a change in tissue sensitivity to zoxazolamine, and (2) a decrease in the plasma decline rate for zoxazolamine.

Partial hepatectomy. Duration of sleep was determined in rats given an injection of hexobarbital Na (75 mg/kg) 48 hr after hepatectomy and 72 hr after cadmium administration (2 mg/kg; i.p.). Sham-operated controls were treated with saline. The data presented in Table 2 indicate that hepatectomy significantly prolonged hexobarbital-sleep time when compared to the sham-operated controls as previously reported by Fouts *et al.* [28]. In addition, cadmium-treated, sham-operated rats slept longer than the sham-operated, saline controls, thus confirming our earlier results. However, cadmium-treated hepatectomized rats did not sleep longer than the corresponding hepatectomized control animals. The data suggest, therefore, that an intact liver is essential for cadmium-induced potentiation of hexobarbital-sleep time.

All animals used in this experiment were allowed to recover and were maintained until day 11 post surgery. Fouts *et al.* [28] have shown that full recovery of the drug-metabolizing enzymes under consideration occurs at about the same time as complete regeneration of the liver mass (10 days). At this time the body and liver weights were determined. The liver weights (not shown) were not different at day 11 after hepatectomy and the liver/body ratios were identical in all experimental groups (Table 2). These data indicate that cadmium treatment does not interfere with regeneration of the liver mass.

Perfused liver studies. Livers were removed from rats 72 hr after treatment with Cd or saline. Rate of disappearance of hexobarbital or rate of appearance of morphine was then determined during a perfusion period of 1 hr. Codeine was used here as a model substrate which is metabolized to an active metabolite, i.e. morphine. The data in Table 3 show that cadmium pretreatment significantly decreased the rate of metabolism of hexobarbital (65 per cent) and also decreased the rate of conversion of codeine to morphine (55 per cent). These results support the hypothesis that the Cd-induced prolongation of drug response results from inhibition of the hepatic drug-metabolizing enzymes.

DISCUSSION

After the acute administration of cadmium, a significant alteration in drug response is observed in the rat. The data presented suggest that the major underlying basis for this phenomenon is inhibition of hepatic drug metabolism, although for certain specific drugs (i.e. zoxazolamine) a change in tissue responsiveness may play a minor role.

This hypothesis is suppored by the observation that the plasma decline of hexobarbital was much reduced in the cadmium-treated rats. While duration of sleep in the cadmium animals was 2·5 times longer than in the controls, the awakening plasma levels of hexobarbital were the same in both groups, indicating that the cadmium effect was the result of an alteration in drug disposition.

Results provided by the partial hepatectomy study indicate that the cadmium effect on hexobarbital sleep is mediated via the liver. Fouts *et al.* [28] have reported that hepatectomy significantly reduces the metabolic capacity of the liver and significantly prolongs sleep time induced by hexobarbital. These authors further report that very little recovery in enzymatic activity occurs within the first 48 hr post surgery. Since partial removal of the liver should decrease the contribution of drug metabolism as a factor in prolonging the duration of drug response, a prolongation of the duration of sleep by cadmium treatment in hepatectomized animals would infer that factors other than drug metabolism (i.e. changes in tissue responsiveness) were operating. The results obtained in our study indicate that the prolonged hexobarbital sleeping time after treatment with cadmium is dependent upon the presence of an intact liver, further suggesting that an alteration in hepatic drug metabolism is the major mechanism by which cadmium potentiates this drug response.

The liver perfusion studies provide additional evidence that cadmium treatment inhibits drug metabolism in the intact liver. In our study, the metabolism of hexobarbital was reduced 65 per cent by cadmium, which correlates with the 2·5 time increase in hexobarbital sleep time observed *in vivo*. To corroborate this finding, the metabolism of codeine was also studied, and a significant reduction (45 per cent) in the metabolic conversion of this substrate to morphine was also found. Preliminary studies (data not presented) have also indicated that cadmium significantly prolongs (2 times) the onset of action for tremorine-induced tremors. Tremorine is converted to the tremorogenic metabolite, oxotremorine, by the liver.

Cadmium induced a prolongation of the pharmacological response to zoxazolamine as observed with hexobarbital. After cadmium treatment, zoxazolamine-paralysis time was increased 185 per cent. Moreover, cadmium-treated animals exhibited significantly higher plasma zoxazolamine levels when sacrificed prior to recovery at a time corresponding to the mean duration of paralysis in control rats. These data alone would suggest that the prolonged zoxazolamine response was the result of a decrease in plasma decline rate which, in turn, is related to a decrease in hepatic drug metabolism. We have also found a 73 per cent decrease in the rate of metabolism in hepatic whole homogenates (unpublished observations). However, when plasma levels of zoxazolamine were measured upon recovery of the paralysis, these levels were lower in the cadmium-treated than in the control animals. This indicates that an increase may have occurred in tissue responsiveness.

Although the data indicate a change in tissue sensitivity with one drug (zoxazolamine) but not the other (hexobarbital), it should be noted that these drugs exert their actions at different sites in the CNS. Hexobarbital depresses activity of the cerebral cortex, while zoxazolamine inhibits neurons in the spinal cord. Gabbiani *et al.* [29] have reported that at 30 days of age cadmium produces ganglionic hemorrhagic lesions in the rat spinal cord, but does not cause similar changes in the cerebrum or cerebellum. It should be noted that the dose of Cd used by Gabbiani *et al.* [29], 6·1 mg/kg, s.c., Cd^{2+}, was considerably higher than our dose, 0·84 mg/kg, Cd^{2+}, i.p. It is altogether possible that changes in the spinal ganglia of the cadmium-treated rat may alter the sensitivity to zoxazolamine without altering the response of the brain to hexobarbital.

The alteration of hexobarbital-sleep time observed after treatment with cadmium is apparently influenced by the route of administration of the cadmium. We have consistently found a prolongation of sleep after the intraperitoneal administration of cadmium acetate [18–20]. Similar findings have been reported in mice by Lewis and Forney [30]. In contrast, Wagstaff [21] has recently reported a decrease in hexobarbital sleep time when cadmium acetate was administered orally in the diet for 15 days. Correlated with this observation, Wagstaff [21] also found a stimulation in the oxidative cleavage of *O*-ethyl-*p*-nitrophenylphenylphosphonothioate (EPN) and the oxidative *O*-demethylation of *p*-nitroanisole. Both the decrease in hexobarbital-sleep time and the stimulation in oxidative drug metabolism were dose related to the concentration of cadmium (100–5000 ppm) in the diet. The reason for the differences in these observations is unknown.

The mechanism by which intraperitoneally administered cadmium inhibits drug metabolism in the liver remains unknown. A recent report by Unger and Clausen [31] indicates that Cd inhibits the activity of P-450 in the mouse. Hadley *et al.* [18] have reported a decrease of 50 per cent in P-450 activity in the male rat.

In summary, treatment with cadmium has been shown to produce a potentiation in pharmacological response to both hexobarbital and zoxazolamine. Preliminary data from additional studies indicate that the major underlying basis for this effect is an inhibition of drug-metabolizing enzyme activity in the liver.

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