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Hypophysial Regulation of Cadmium-Induced Depression of the Hepatic Monooxygenase System in the Rat

E. M. K. LUI[1] AND G. W. LUCIER

Laboratory of Organ Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

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SUMMARY

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The effect of acute cadmium treatment on the hepatic monooxygenase activities of adult rats was assessed 72 hr following a single dose of $CdCl_2$ (1.0 or 2.0 mg/kg); marked reduction in cytochrome P-450 contents and ethylmorphine *N*-demethylation activity was observed only in the males. Hypophysectomy at 50 days of age enhanced the cadmium responsiveness in the subsequent 80-day-old male and female rats, suggesting a protective effect of the pituitary against the hepatotoxic effects of cadmium in adult rats. However, hypophysectomy of male and female rats at 20 days of age abolished their hepatic cadmium responsivity in adult life. It appears that pituitary influences are required for a period between 20 and 50 days of age for the development of hepatic cadmium responsiveness. Therefore, we postulate that pituitary influences with cadmium-sensitizing and cadmiun-antagonizing properties may be functioning in both male and female rats during the pre- and postpubertal periods, respectively. Pituitary influences also altered the hepatic disposition of cadmium; however, the effects of the pituitary on hepatic cadmium responsiveness are probably not due to its effect on hepatic cadmium contents. Microsomes isolated from livers of untreated male or female rats which were either hypophysectomized or sham-operated showed similar susceptibility toward the *in vitro* inhibitory action of cadmium on the hepatic monooxygenase system.

INTRODUCTION

Cadmium has been recognized as a toxic environmental pollutant (1, 2). One of the toxic manifestations following acute cadmium treatment is the depression of HMO[2] activities. This cadmium-responsiveness is both age- and sex-related; marked reduction in enzyme activities is observed in adult males but not adult females (3, 4) or immature rats of either sex (3, 5).

The HMO system is responsible for the oxidative metabolism of endogenous as well as exogenous chemicals. The enzyme system of immature rats displays no sex differences, but this enzyme system undergoes sexual differentiation in male rats during the pubertal period. Adult male characteristics are fully expressed by 7–8 weeks postpartum (6, 7) and the postpubertal enzyme activities are usually higher in males than in females. The higher levels of enzyme activity are believed to be maintained, in part, by testicular androgens (8, 9). Accordingly, it has been postulated that the cadmium-induced depression of HMO activity in adult male rats may be related to a reduction of circulating androgen levels secondary to testicular toxic effects of cadmium (4). Recent studies from our laboratory suggest that differences in the cadmium responsiveness of the HMO system may be related to certain sex-related characteristics of the enzyme system, since adult male rats which have received pharmacological doses of diethylstilbestrol or testosterone propionate during the neonatal period possess HMO with "feminine" characteristics and display a lack of cadmium-responsiveness (feminized) (3). Since recent studies suggest that the hepatic effects of gonadal hormones and the development and maintenance of sex-dependent characteristics of HMO in the rat are mediated by the pituitary (10, 11), we have examined in detail the role of pituitary glands during the pre- and postpubertal period in the expression of cadmium responsiveness of HMO. Data from the present investigation suggest that pituitary influences may involve both cadmium-sensitizing and cadmium-antagonizing factors. Moreover, these studies indicate that sex differences in

[1] Present address: Department of Pharmacology, Faculty of Medicine, University of Western Ontario, London, Ontario N6A 5Cl, Canada.

[2] The abbreviations used are: HMO, hepatic monooxygenase; ED, ethylmorphine *N*-demethylation.

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the cadmium susceptibility of the HMO may be due to quantitative differences in these pituitary-dependent factors and/or sex-related differences in the hepatic sensitivity toward pituitary influences in the rat.

METHODS

Animals and treatments. Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used throughout these studies. The animals were kept four per cage and housed on corncob bedding with free access to food (NIH Feed-31) and deionized water unless otherwise stated. The animals were kept at room temperature (21°) and exposed to alternate 12-hr light and darkness cycles. Animals were hypophysectomized at day 20 or 50 of age and received 0.9% NaCl solution (saline) with 5% glucose as drinking water during the first 10 days following surgery. Sham-operated animals received the same treatment. To assess the hepatic response to acute cadmium treatment, 77- or 110-day-old rats received a single i.p. injection of $CdCl_2$ (1.0 or 2.0 mg/kg) in a volume of 2 ml/kg of body weight. Control animals received comparable volumes of saline. The animals were killed and examined 72 hr following cadmium treatment.

Tissue preparation. Rats were killed by decapitation and the livers were immediately removed and weighed. Livers were minced with scissors and 1.0-g liver samples were stored at −70° for cadmium residue analysis. Liver samples were homogenized in a Potter-Elvehjem glass homogenizer equipped with a motor-driven Teflon pestle using sufficient ice-cold 1.15% KCl-0.05 M Tris buffer (pH 7.4) to produce a 20% (w/v) homogenate. Homogenates were centrifuged at 9,000 × *g* for 10 min; the resulting supernatant was centrifuged at 105,000 × *g* for 60 min. The resultant microsomes were resuspended in 0.15 M Tris buffer (pH 7.4) so that 1.0 ml of microsomal suspension was equivalent to 0.5 g of wet liver. An aliquot of the microsomal suspension was stored at 4° and used within 2 hr of preparation for enzyme assays. The remaining microsomal suspension was resedimented at 105,000 × *g*, and the washed pellet was resuspended in 0.15 M Tris buffer, pH 7.4 (approx. 10 mg of protein/ml of microsomal suspension), and used for hemoprotein determination.

Biochemical analyses. Hepatic microsomal ethylmorphine *N*-demethylation was determined as described previously (3). The activity of NADPH-cytochrome *c* reductase was measured at 37° according to the method of Glende (12). Oxidized cytochrome *c* (horse heart, Type II) (0.1 μmole) was added to an incubation mixture containing microsomes (5–10 μg of protein), potassium cyanide (1.0 μmole), nicotinamide (80 μmoles), and NADPH (0.3 μmole) in 0.067 M phosphate buffer, pH 7.4. The total volume of the reaction mixture was 3.0 ml. The enzyme activity was expressed as nanomoles of cytochrome *c* reduced per milligram of protein per minute. The activity of ethoxyresorufin O-deethylation was determined by the method of Burke *et al.* (13). The cytochrome P-450 content was measured by the carbon monoxide difference spectra after reduction with dithionite (14). Microsomal protein was determined by the procedure of Lowry *et al.* (15), using bovine serum albumin as the standard.

Cadmium residue analysis was accomplished by ashing liver samples at 450° for 24 hr, and cadmium concentrations were determined by atomic absorption spectrometry.

Statistical analysis of significance was established at $p < 0.05$ with Student's *t*-test for two-sample means based on independent samples.

For the determination of hepatic microsomal cadmium contents, animals were injected with a single dose of $CdCl_2$ (2.0 mg/kg with 4 μCi of $^{109}CdCl_2$) and killed 72 hr later. Microsomal preparations were prepared as described above. The radioactivity of 1-ml samples was counted in a γ-counting spectrometer (Biogamma II; Beckman Instrument, Inc., Fullerton, Calif.).

Chemicals. Cadmium chloride and sodium hydrosulfite were purchased from Fisher Scientific Company (Fairlawn, N. J.); ^{109}Cd (carrier-free) was obtained from New England Nuclear Corporation (Boston, Mass.); ethylmorphine HCl and NADPH were obtained from Merck & Company, Inc. (Rahway, N. J.), and Sigma Chemical Company (St. Louis, Mo.), respectively. All other chemicals were obtained from the J. R. Baker Chemical Company (Phillipsburg, N. H.).

RESULTS

Effect of postpubertal hypophysectomy on sex differences of HMO activity. Sex differences in HMO activity of rats are fully developed by 50 days of age (7). Accordingly, 50-day-old rats were hypophysectomized and the HMO activity was examined 30 days later. There were small but insignificant sex differences in the cytochrome P-450 contents in microsomes isolated from livers of adult rats (Table 1). However, the hemoprotein contents were significantly elevated in both male and female rats by hypophysectomy. The rates of *in vitro* ED of intact adult male rat livers were approximately 3- to 4-fold higher than those of intact females when the results were expressed either on the basis of microsomal protein or nanomoles of cytochrome P-450. Both of these parameters of ED activity were decreased by prior hypophysectomy of adult male rates, whereas only the specific activity of ED (based on microsomal protein) was altered (increased) in the female. No sex differences in these parameters were seen in hypophysectomized rats. Microsomal NADPH-cytochrome *c* reductase activities, which exhibited no sex differences in adult rats, were reduced by one-half after hypophysectomy of either male or female rats.

Hypophysectomized animals were also examined 60 days after surgery. Changes in the HMOs of these animals were similar to those detected 30 days following hypophysectomy (data not shown). These data reveal that postpubertal hypophysectomy abolishes sex differences in the HMO parameters that were investigated in this study.

Postpubertal hypophysectomy and the response of HMO system to cadmium in the male rat. Since pituitary glands appear to regulate expression of postpubertal sex differences in the HMO system, we have investigated the involvement of pituitary glands in the sex-dependent modulation of cadmium effects on this enzyme system. Rats were examined 72 hr following cadmium treatment. Preliminary time-course studies had indicated that *in*

TABLE 1

Effect of postpubertal hypophysectomy[a] on the hepatic monooxygenase system of 80-day-old rats[b]

	Cytochrome P-450	Ethylmorphine *N*-demethylation		NADPH-cytochrome *c* reductase
	nmoles/mg protein	*nmoles HCHO/min/mg protein*	*nmoles HCHO/min/nmole cytochrome P-450*	*nmoles/min/mg protein*
Male				
Sham-operated	0.64 ± 0.08 (13)	10.20 ± 1.80 (13)	16.0	45.5 ± 1.8 (6)
Hx-50	0.90 ± 0.11[c] (11)	5.60 ± 1.11[c] (11)	6.2	18.9 ± 3.5[c] (6)
Female				
Sham-operated	0.55 ± 0.10 (15)	3.19 ± 0.51 (14)	5.8	49.3 ± 4.5 (6)
Hx-50	0.90 ± 0.16[c] (15)	4.52 ± 0.36[c] (15)	5.0	24.1 ± 3.0[c] (6)

[a] Rats were hypophysectomized at 50 days of age (Hx-50) and examined 30 days later.
[b] Each value is the mean ± standard deviation of the mean from the number of animals appearing in parentheses.
[c] Significantly different from sham-operated controls of the same sex at least at $p < 0.05$.

vitro hepatic enzyme activities were maximally affected at this time (data not shown). Treatment of adult male rats with 2 mg/kg of $CdCl_2$ resulted in a 42% reduction in hepatic microsomal cytochrome P-450 levels, although no significant decrease in this parameter was observed at a cadmium dosage of 1 mg/kg (Fig. 1). The rate of ED, whether expressed on the basis of microsomal protein or nanomoles of cytochrome P-450, was reduced by either dose of cadmium. In contrast, NADPH-cytochrome *c* reductase activities of hepatic microsomes from adult rats were not altered by cadmium exposure (data not shown). Furthermore, cadmium treatment did not alter the rate of microsomal ethoxyresorufin *O*-deethylation; the specific activities for the control and cadmium-treated controls were 0.12 and 0.13 nmole/min/mg of protein, respectively.

Data presented in Fig. 1 also illustrate the effects of hypophysectomy on hepatic cadmium responsiveness in adult male rats. Exposure of hypophysectomized male rats to 1.0 mg/kg of $CdCl_2$ resulted in a decrease in the cytochrome P-450 contents (43%) and the specific activities of ED (48%). However, no significant increase in the cadmium-induced reduction of HMO activities was detected when the cadmium dosage was increased to 2.0 mg/kg. These data suggest that the pituitary is not required after 50 days of age for expression of Cd effects on the HMO system.

Postpubertal hypophysectomy and the response of HMO system to cadmium in the female rat. In contrast to males, neither female hepatic cytochrome P-450 nor

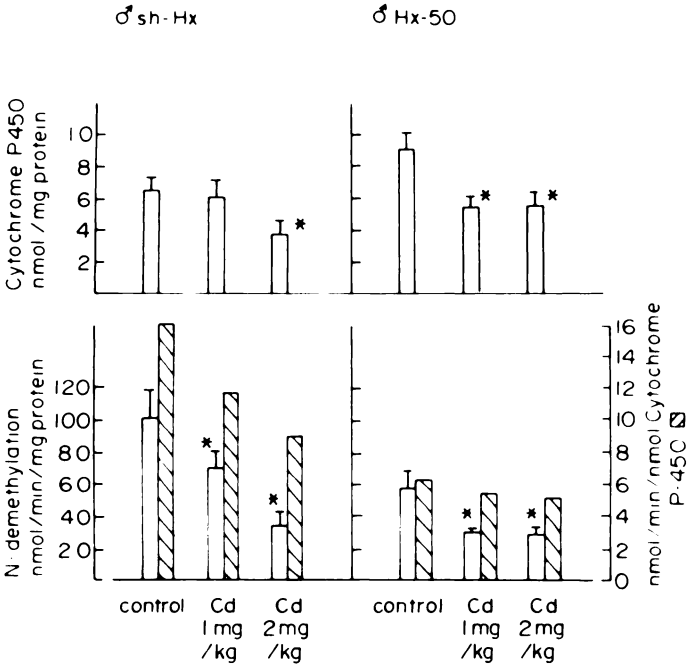

FIG. 1. *Effect of postpubertal hypophysectomy (Hx) on the cadmium-induced reduction in HMO activity of the male rat*

Rats were hypophysectomized at 50 days of age and examined 30 days later. Cadmium responsiveness was assessed by administering a single dose of $CdCl_2$ (i.p.) to 77-day-old rats, and rats were examined 72 hr later. Control animals received comparable volumes of saline. The values represent the mean enzyme activities. The line represents the standard deviation of the mean from six to eight individual rats, and an *asterisk* (*) signifies statistically significant differences from control values at least at $p < 0.05$.

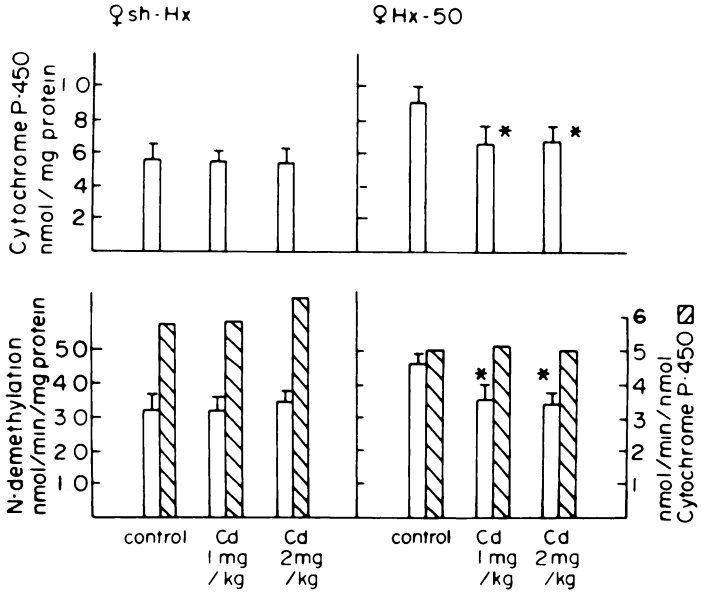

FIG. 2. *Effect of postpubertal hypophysectomy (Hx) on the cadmium-induced reduction in HMO activity of the female rat*

Rats were hypophysectomized at 50 days of age and examined 30 days later. Cadmium responsiveness was assessed by administering a single dose of $CdCl_2$ (i.p.) to 77-day-old rats, and the rats were examined 72 hr later. Control animals received comparable volumes of saline. The values represent the mean enzyme activities. The line represents the standard deviation of the mean from six to eight individual rats, and an *asterisk* (*) signifies statistically significant differences from control values at least at $p < 0.05$.

ED were affected by acute cadmium treatment (Fig. 2). However, hypophysectomy at 50 days of age appears to confer cadmium-susceptibility to the HMO system of adult female rats. Treatment with a cadmium dosage of either 1.0 or 2.0 mg/kg resulted in approximately 30% reduction in both cytochrome P-450 content and ED activity of hypophysectomized females (Fig. 2). However, the rate of ED, when expressed on the basis of nanomoles of cytochrome P-450 or NADPH-cytochrome *c* reductase activity, was not affected by treatment of these animals with cadmium. Therefore, the cadmium-induced depression of ED specific activity in the females could be accounted for by the decrease in cytochrome P-450 levels. It was also noted that the hepatic responsiveness to cadmium treatment in hypophysectomized female rats persisted when they were examined 60 days following surgery (data not shown). These data suggest that the presence of pituitary glands protects female rats against the hepatotoxic effects of cadmium.

Prepubertal hypophysectomy and the hepatic cadmium response in male and female rats. Since the HMO system of immature rats is not sexually differentiated and is not susceptible to acute cadmium treatment (3, 5), it was of interest to determine whether prepubertal hypophysectomy would prevent the development of hepatic cadmium-responsiveness. In this study, 20-day-old male and female rats were hypophysectomized and their hepatic cadmium response was assessed 60 days later. Data presented in Table 2 reveal that prepubertal hypophysectomy prevented the expression of sex differences in the HMO system of subsequent adult rats. Furthermore, hepatic cytochrome P-450 and ED activity were not significantly depressed by cadmium treatment of these animals. These data suggest that the pituitary is required during the prepubertal period for the development of hepatic sensitivity to cadmium in the subsequent adult animal.

In vitro effect of cadmium on the monooxygenase activity of rat liver microsomes. It has been reported that addition of cadmium to hepatic microsomes isolated from adult male rats results in inactivation of cytochrome P-450 and decreases in the rate of *N*-demethylation (16). To evaluate further the effect of hypophysectomy on the hepatotoxic action of cadmium *in vivo*, the *in vitro* effect of cadmium was also investigated. The rate of *in vitro* *N*-demethylation was reduced in the presence of 1×10^{-6} to 1×10^{-4} M cadmium in a concentration-dependent manner (Table 3), regardless of the sex or surgical procedures performed on the rats prior to microsome preparation. Furthermore, the rate of *O*-deethylation of ethoxyresorufin (which is unaffected by *in vivo* cadmium treatment) in hepatic microsomes of adult male rats was reduced by 25 and 70% in the presence of 1×10^{-5} and 1×10^{-4} M cadmium, respectively. Therefore, it appears that the reduction in HMO activities following cadmium treatment is not due to direct inhibitory actions.

Body weight, liver weight, and hepatic cadmium contents. Since cadmium is preferentially taken up by the liver following acute treatment (17), possible sex differences in hepatic cadmium contents were investigated.

Seventy-two hours following a single dose of $CdCl_2$ (2.0 mg/kg), the cadmium contents of the livers of adult male rats were higher than those of females (Tablse 4 and 5). However, when total hepatic cadmium was expressed on the basis of the amount of cadmium received, no sex difference in cadmium contents could be detected. Prior hypophysectomy at 50 days of age appears to increase the ability of the liver from both male and female rats to accumulate cadmium, since hepatic cadmium levels when expressed on the basis of either liver weight or total

TABLE 3

Effect of cadmium on the in vitro N-demethylation of ethylmorphine[a] from microsomes isolated from liver of sham-operated and hypophysectomized[b] rats

	% Decrease[c] in ED activity with cadmium concentrations of		
	1×10^{-6} M	1×10^{-5} M	1×10^{-4} M
Male			
Sham-operated	17	62	95
Hypophysectomized	5	40	77
Female			
Sham-operated	33	76	83
Hypophysectomized	16	47	84

[a] ED activity was measured as indicated under Methods, except that suitable concentrations of cadmium were incubated with the microsomes, cofactors, and substrates for 5 min before the addition of NADPH.

[b] Rats were hypophysectomized at 50 days of age and killed 30 days later.

[c] Each value represents the mean of four determinations.

TABLE 2

Effect of prepubertal hypophysectomy on the cadmium responsiveness of the hepatic monooxygenase of 80-day-old rats[a]

	Cytochrome P-450		Ethylmorphine *N*-demethylation	
	0 $CdCl_2$	2.0 mg/kg $CdCl_2$	0 $CdCl_2$	2.0 mg/kg $CdCl_2$
	nmoles/mg protein		*nmoles HCHO/min/mg protein*	
Male				
Sham-operated	0.63 ± 0.07	0.35 ± 0.10[b]	11.10 ± 1.50	3.90 ± 0.9[b]
Hx-50	0.89 ± 0.14	0.79 ± 0.10	4.90 ± 0.50	4.35 ± 0.45
Female				
Sham-operated	0.55 ± 0.10	0.53 ± 0.09	3.20 ± 0.45	3.45 ± 0.31
Hx-50	0.90 ± 0.16	0.91 ± 0.15	4.42 ± 0.23	4.06 ± 0.27

[a] Rats were injected with a single dose of $CdCl_2$ (2.0 mg/kg, i.p.) at 77 days of age and examined 72 hr later. The values represent the mean ± standard deviation for six animals.

[b] Significantly different from saline-treated controls at least at $p < 0.05$.

[c] 20-day-old rats were hypophysectomized and examined 60 days later.

TABLE 4

Body weight, liver weight, and hepatic cadmium content of 80-day-old intact or hypophysectomized male rats[a]

	Sham-operated		Hx-50[b]		Hx-20[b]	
	Saline	Cadmium[c]	Saline	Cadmium[c]	Saline	Cadmium[c]
Body wt. (g)	346 ± 17	308 ± 34	154 ± 7	151 ± 17	68 ± 4	68 ± 4
Liver wt. (g)	13.5 ± 1.6	10.8 ± 1.1	5.1 ± 0.7	4.4 ± 0.6	2.6 ± 0.4	2.5 ± 0.2
(liver wt.)/(body wt.) × 100	3.9 ± 0.2	3.3 ± 0.3[d]	3.3 ± 0.4	2.9 ± 0.3	3.8 ± 0.4	3.7 ± 0.4
Cadmium contents (μg/g of liver, wet wt.)	ND[e]	17.8 ± 1.3	ND	24.1 ± 2.4[f]	ND	18.2 ± 1.3
Liver cadmium/cadmium administered	ND	0.56	ND	0.70	ND	0.68

[a] Each value (mean ± standard error) was derived from 6–10 animals.
[b] Rats were hypophysectomized at either 20 (Hx-20) or 50 (Hx-50) days of age.
[c] 77-day-old rats were injected with a single dose of $CdCl_2$, 2 mg/kg i.p. (980 μg of cadmium per 2 mg of $CdCl_2$) and examined 72 hr later.
[d] Significantly different from saline-treated controls at least at $p < 0.05$.
[e] ND, not detectable, the limit of detection being 0.5 μg/g of liver.
[f] Significantly different from sham-operated controls at $p < 0.05$.

amount of cadmium administered were higher in hypophysectomized than in sham-operated animals (Tables 4 and 5). However, the hepatic microsomal cadmium concentrations of hypophysectomized or sham-operated male and female rats were similar, and the mean microsomal cadmium concentrations of these animals ranged from 0.038–0.044 μg/mg of protein. These data suggest that sex differences in the response of the HMO system to cadmium is not related to sex-specific tissue accumulation of cadmium. Acute cadmium treatment caused a significant decrease in the liver weight/body weight ratio in sham-operated males, but not in females. This ratio did not change in hypophysectomized male rats.

DISCUSSION

Our studies demonstrate the importance of pituitary functions in determining the responsiveness of the HMO enzyme system to cadmium. On the basis of our observations, we have proposed a model which illustrates the involvement of pituitary influences in both the development and the subsequent modulation (antagonism) of hepatic cadmium responsiveness of the HMO system in the rat (Fig. 3). These pituitary influences are referred to as "pituitary-dependent factors," although their characteristics and mechanism of action have not been established. It appears that pituitary influences are required for a period between 20 and 50 days of age for the development of cadmium responsiveness, since hypophysectomy at 20 days of age prevented the development of cadmium responsiveness in adult male rats (Fig. 3B and C). Moreover, hypophysectomy at day 50 confers cadmium responsiveness to livers of females, whereas hypophysectomy at day 20 does not confer responsiveness to the subsequent adult female. However, the cadmium responsivity that develops between day 20 and day 50 is not fully expressed if the pituitary glands are also present during the postpubertal period, since postpubertal hypophysectomy (day 50) increased the sensitivity of the HMO system toward cadmium over that of sham-operated controls (Fig. 3A and B). Therefore, we postulate that "pituitary factors" or influences with cadmium-sensitizing and cadmium-antagonizing properties may regulate the sex differentiation of the hepatic response to cadmium.

The reason for the divergent effects of the pituitary is unclear, although they appear to have different modes of action. The increase in cadmium responsiveness following postpubertal hypophysectomy (day 50) may reflect a

TABLE 5

Body weight, liver weight, and hepatic cadmium content of 80-day-old intact or hypophysectomized female rats[a]

	Sham-operated		Hx-50[b]		Hx-20[b]	
	Saline	Cadmium[c]	Saline	Cadmium[c]	Saline	Cadmium[c]
Body wt. (g)	249 ± 30	258 ± 23	120 ± 4	126 ± 4	69 ± 4	75 ± 5
Liver wt. (g)	9.2 ± 2.0	10.1 ± 1.9	4.5 ± 0.2	4.0 ± 0.5	2.8 ± 0.3	2.6 ± 0.2
(liver wt.)/(body/wt.) × 100	3.9 ± 0.4	3.9 ± 0.8	3.5 ± 0.1	3.2 ± 0.3	3.9 ± 0.2	3.7 ± 0.3
Cadmium contents (μg/g liver, wet wt.)	ND[e]	13.7 ± 2.0	ND	21.8 ± 2.8[f]	ND	18.9 ± 2.4
Liver cadmium/cadmium administered	ND	0.55	ND	0.72	ND	0.71

[a] Each value (mean ± standard error) was derived from 6–10 animals.
[b] Rats were hypophysectomized at either 20 (Hx-20) or 50 (Hx-50) days of age.
[c] 77-day-old rats were injected with a single dose of $CdCl_2$, 2 mg/kg i.p. (980 μg cadmium per 2 mg of $CdCl_2$) and examined 72 hr later.
[d] Significantly different from saline-treated controls, at least at $p < 0.05$.
[e] Not detectable, the limit of detection being 0.5 μg/g liver.
[f] Significantly different from controls, at least at $p < 0.05$.

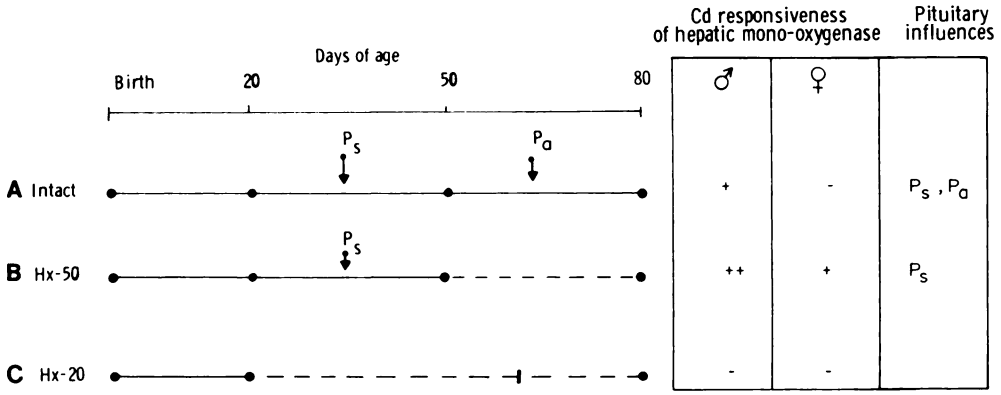

FIG. 3. *Hypothetical model of the pituitary regulation of hepatic cadmium responsiveness in intact (A), postpubertally hypophysectomized (B), and prepubertally hypophysectomized (C) male and female rats*

The presence and absence of pituitary glands during development are indicated by —— and - - -, respectively. *Pa* and *Ps*, the actions of pituitary cadmium-antagonizing factor and cadmium-sensitizing factor, respectively. Based on the findings presented in Figs. 1 and 2, the degree of hepatic cadmium responsiveness is expressed in an arbitrary scale of −, + and ++. *Hx*, hypophysectomy.

modulating role of the pituitary, since cadmium responsiveness in male rats was fully developed at 50 days of age. However, the pituitary-dependent development of cadmium responsivity during development (days 20–50) is probably due to the pituitary-mediated induction of some permanent changes in liver or other endocrine interactions involving peripheral endocrine organs that have impact on liver cell regulatory processes. It appears that the pituitary plays a similar role in both male and female rats in the regulation of hepatic cadmium responsiveness; therefore, the sexual dimorphism in cadmium susceptibility may be attributable to quantitative differences in the levels of these pituitary-dependent factors, and/or to sex differences in the sensitivity of the liver to these "factors."

Discussion of the mechanisms underlying the pituitary-dependent regulation of hepatic cadmium responsiveness must be considered in order to understand the biochemical basis for the hepatic response to cadmium. Recent studies have dealt with specific components of the HMO system with unique sensitivity to cadmium (16, 18–20). Hepatic cytochrome P-450 is thought to exist in six to eight different forms in the rat (21, 22). Although the total cytochrome P-450 contents in immature and mature rats of either sex are similar, their turnover characteristics as well as the abundance of specific forms of this hemoprotein are both age- and sex-related (21, 23). Several reports in the literature provide evidence for the theory that cadmium exerts selective effects on different forms of cytochrome P-450. (a) Hemoprotein turnover studies reveal that the increase in total cytochrome P-450 content following hypophysectomy in both male and female rats is accompanied by an increase in the amounts of slowly-turning-over hemoproteins which normally exist in higher concentration in males than in females (11, 18). Our recent studies as well as others have shown that cadmium treatment preferentially reduces the contents of the slow-phase components (18, 20). (b) Cadmium-induced reduction in the rate of oxidative metabolism of the Type I substrate, ethylmorphine, is greater than those of the Type II substrate, aniline, in the adult male rat (15). However, ethoxyresorufin is also a Type I substrate and the demethylation reaction is unaffected by cadmium. Therefore, selective effects of cadmium on hemoproteins involves more than distinction between Type I and Type II substrates. It is possible that the pituitary-dependent factors postulated in this study may act by regulating the availability of cadmium-sensitive cytochrome P-450 in adult rats.

There is the possibility that hepatic effects of cadmium are related to effects of cadmium on androgen levels. It is known that androgens are a positive modulator of hepatic *N*-demethylation but not cytochrome P-450 of adult male rats, since postpubertal castration results in reduction in *N*-demethylation activity but no changes in the total contents or the turnover profile of cytochrome P-450 (21, 22). Cadmium is also a testicular toxin; its chemical castration effect (reduced circulating androgen levels; ref. 24) could reduce *N*-demethylation activity without altering cytochrome P-450 contents. However, the androgen component in the hepatic response to cadmium is probably of limited significance, since postpubertal hypophysectomy (which markedly depresses androgen levels) did not decrease the cadmium responsiveness of the HMO system. In fact, hypophysectomy actually increased the hepatic response to cadmium. Moreover, it has been reported that castration of adult male rats fails to offer complete protection against the ability of cadmium to depress HMO activities.

The effect of postpubertal hypophysectomy on the HMO system of either male or female rats cannot be produced by gonadectomy, adrenalectomy, or thyroparathyroidectomy of adult animals (11). These findings suggest that follicle-stimulating hormone, luteinizing hormone, adrenocorticotropic hormone, and thyroid-stimulating hormone do not play a major role in the modulation of cadmium responsiveness of the enzyme system. However, streptozotocin-induced diabetes in both adult male and female rats resulted in changes in the HMO system that are identical with (25) those resulting from postpubertal hypophysectomy as reported in the present study. The possibility, therefore, exists that insulin might play an important role in regulating the hepatotoxic effect of cadmium in the rat.

The pituitary also influences hepatic disposition of cadmium; we observed an apparent correlation between the elevation of hepatic cadmium contents and the increase in hepatic cadmium responsiveness by prior postpubertal hypophysectomy (Table 4). However, the HMO activity of adult female rats remained resistant to cadmium treatment despite an increase in the dosage of cadmium to 3.0 mg/kg; and cadmium analysis studies revealed that the hepatic cadmium contents of these animals were similar to those of postpubertally hypophysectomized females receiving a 2-mg/kg dose of cadmium[3]. Furthermore, our studies showed that the decrease in enzyme activities following cadmium treatment was probably not a result of the direct action of cadmium on the enzyme components, since microsomes isolated from livers of untreated male or female rats which were either hypophysectomized or sham-operated showed similar susceptibility toward the *in vitro* inhibitory action of cadmium (Table 3). Moreover, the concentration of cadmium *in vitro* required to depress enzyme activities is much higher than the microsomal cadmium contents of intact males 72 hr following a single dose of cadmium (2.0 mg/kg).

Our studies suggest that cadmium exerts its sex- and age-dependent effects on the HMO system by a mechanism that apparently involves sex differentiation of liver sensitivity to cadmium. Although we have not elucidated the biochemical mechanisms underlying the action of cadmium, expression of cadmium sensitivity does not require an intact pituitary in adult rats, nor do circulating androgens play a major role in modulating sensitivity of the HMO system to cadmium. However, the development of sensitivity to cadmium is dependent on pituitary influences between 20 and 50 days of age. These pituitary influences act by conferring permanent changes to the liver or by initiating a regulatory process involving the peripheral endocrine system such that once initiated this regulatory process no longer requires pituitary action.

[3] E. M. K. Lui and G. W. Lucier, unpublished observations.

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Send reprint requests to: Dr. G. W. Lucier, Laboratory of Organ Function and Toxicology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N. C. 27709.