MOLECULAR PHARMACOLOGY, 22:795–802

Endocrine Regulation of Cadmium-Sensitive Cytochrome P-450 in Rat Liver

EDMUND M. K. LUI,[1] SHELLEY R. SLAUGHTER, RICHARD M. PHILPOT, AND GEORGE W. LUCIER

Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

Received January 28, 1982; Accepted June 24, 1982

SUMMARY

The biphasic disappearance of labeled heme from microsomal CO-binding particles obtained from livers of adult male and female rats was studied in relation to endocrine status and cadmium treatment. Levels of the slow-phase (SP) component were greater in males than in females, although no sex differences were evident in levels of the fast-phase (FP) component. Cadmium (Cd) treatment (2 mg/kg i.p.) of male rats at 48 hr prior to [^{3}H]δ-aminolevulinic acid injection decreased the FP and SP components by 31% and 68%, respectively. Total hepatic cytochrome P-450 was also decreased by Cd. This was accompanied by a reduction in the half-life of the FP but not of the SP hemoprotein; no such alteration by Cd was seen in females. Hypophysectomy (Hx) of male and female rats resulted in a preferential increase in the SP component which could be counteracted by Cd treatment. These data suggest that the responsiveness of cytochrome P-450 to Cd was associated with the relative abundance of the SP hemoproteins. The effect of Cd on hepatic cytochrome P-450 was further characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of hepatic microsomes. Treatment of adult male rats with Cd reduced the intensity of the staining of several proteins, most notably proteins with molecular weights of 49,000 (Band *A*), 54,000 (Band *B*), and 62,000 (Band *C*). In females these proteins are less concentrated and the effects of Cd are less pronounced than in males. However, Hx of male and female rats increased the staining intensities of these bands, an increase which could be reversed by treatment with Cd. Therefore, the concentrations of hepatic cytochrome P-450 and protein Bands *A*, *B*, and *C* are affected in the same direction by gender, Hx, and Cd treatment. Cd treatment also resulted in an increase in hepatic microsomal heme oxygenase activity of sham-operated or hypophysectomized male and female rats at 24 and 48 hr following treatment. The apparent lack of correlation between the increase in heme oxygenase activity and the Cd-mediated decrease of cytochrome P-450 suggests insignificant involvement of heme oxygenase in the sex- and pituitary-dependent response of cytochrome P-450 to Cd.

INTRODUCTION

The depression of certain hepatic microsomal monooxygenase activities in adult male rats following acute Cd[2] treatment has been well documented (1–3). The Cd responsiveness of this enzyme system is sex-related; a marked reduction in enzyme activity is observed in adult male rats but not in female rats (4, 5). Recently, we have shown that hepatic Cd responsiveness can also be influenced by pituitary function. Postpubertal Hx enhances the Cd sensitivity of the monooxygenase system in both male and female rats and abolishes the sex differences in Cd responsiveness (6). Although the mechanism of Cd-mediated decreases in the hepatic monooxygenase of the male rat is not clear (2, 7, 8), there is evidence that the sex- and pituitary-dependent responsiveness of this enzyme system to Cd are a consequence of changes in certain characteristics of cytochrome P-450, the terminal oxidase of the monooxygenase system (6).

Hepatic microsomal cytochrome P-450 in rats exhibits biphasic turnover characteristics. Distinct FP and SP components are revealed by following the disappearance of labeled heme from microsomal CO-binding particles obtained from rat liver (9–11). The number of hemoprotein components detected by this method is uncertain. The possibility also exists that the CO-binding particles contain precursors of cytochrome P-450. Results from other studies using electrophoretic, immunological, and chromatographic techniques have implicated the pres-

[1] Present address, Department of Pharmacology and Toxicology, Faculty of Medicine, University of Western Ontario, London, Ont. N6A 5C1, Canada.

[2] The abbreviations used are: Cd, cadmium; Hx, hypophysectomy; FP, fast-phase; SP, slow-phase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

0026-895X/82/060795-08$02.00/0

ence of at least six different cytochrome P-450 species in hepatic microsomes of male rats (12–14). Although the total cytochrome P-450 content of hepatic microsomes is similar in adult male and female rats, the turnover characteristics, as well as the relative abundance of certain forms of cytochrome P-450, are sex-related (9, 10, 15, 16).

Cd is known to exert selective inhibitory effects on different hepatic monooxygenase activities of adult male rats. For example, the oxidative metabolism of substrates such as ethylmorphine, 3-zoxazolamine, and hexobarbital is decreased by Cd to a much greater extent than is that of aniline (1, 3, 5, 7, 8). Moreover, ethoxyresorufin *O*-deethylation is not altered by Cd treatment (6). In an attempt to determine whether Cd alters the concentration of certain forms of cytochrome P-450, we have investigated the Cd responsiveness of hepatic cytochrome P-450 by analysis of hemoprotein turnover and SDS-PAGE profiles of hepatic microsomes as a consequence of gender and postpubertal Hx. Data from this study suggest that Cd responsiveness is associated with levels of the SP hemoprotein(s) as well as that of three microsomal protein bands (as resolved by SDS-PAGE). Since the hepatic microsomal heme oxygenase constitutes the rate-limiting step in the degradation of heme, we have also examined the possible role of this enzyme in the pituitary regulation of sex-dependent Cd responsiveness of cytochrome P-450.

MATERIALS AND METHODS

Animals and treatment. Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used in all experiments. Animals were housed on corncob bedding with free access to food (NIH feed-31) and deionized water unless otherwise stated. Rats were hypophysectomized at 50 days of age and received 0.9% NaCl solution with 5% glucose as drinking water ad libitum during the first 10 days following surgery. Sham-operated animals received the same diet. To examine the effect of acute Cd treatment on the SDS-PAGE profile of hepatic microsomes, 78-day-old rats received injections of a single dose of $CdCl_2$ (2 mg/kg i.p.) in a volume of 2 ml/kg of body weight. Control animals received a corresponding volume of 0.9% NaCl solution. The animals were killed and examined 48 hr following treatment. To assess the effect of acute Cd treatment on the turnover rates of hepatic cytochrome P-450, rats received a single dose of [3,5-^{3}H]δ-aminolevulinic acid (70 μCi/kg of body weight, specific activity 960 μCi/nmole). The labeled precursor was dissolved in 0.9% NaCl solution and injected via the tail vein in a volume of 1 ml/kg of body weight 48 hr following Cd or saline treatment. Animals were killed at various times after δ-aminolevulinic acid injection.

Tissue preparation. Rats were decapitated and the livers were removed and weighed. Microsomal fractions were isolated from liver minces (3-g samples) as described previously (5). In experiments involving the electrophoresis of microsomal proteins, the washed hepatic microsomes were suspended in 0.25 M sucrose solution and stored at −20°. This storage condition did not have any apparent effect on the SDS-PAGE profiles of microsomes. In cytochrome P-450 turnover studies, the washed microsomal pellets were layered with 2 ml of 0.1 M phosphate buffer (pH 7.4) and stored at −70° for 3–7 days before use.

Preparation of CO-binding particles. Hepatic microsomal pellets were thawed at room temperature immediately before use and were suspended in 10 ml of 0.1 M phosphate buffer (pH 7.4). The microsomal suspensions were incubated with 20 mg of steapsin at 37° for 60 min under a nitrogen atmosphere to solubilize the cytochrome b_5 fraction. The microsomal suspension was then centrifuged at 165,000 × *g* for 60 min and the resulting pellet was resuspended in 3 ml of 0.1 M phosphate buffer. Radioactivity in resuspended microsomal fractions was determined by liquid scintillation counting using 10 ml of scintillation fluid (Aquasol; New England Nuclear Corporation, Boston, Mass.).

SDS–PAGE. SDS-PAGE of hepatic microsomes was performed on slab gels (1.5 mm thick) as described by Laemmli (17). Microsomal samples equivalent to approximately 40 μg of protein were electrophoresed first at 25 mamp for 1 hr through the stacking gel. Electrophoresis was continued through the gel at 40 mamp for 4.5–5 hr. Gels were stained and destained by using a modification of the method of Weber and Osborn (18). The gels were fixed overnight in a solution of methanol/acetic acid/water (4.5:1:4.5, v/v) and then stained with 0.25% Coomassie blue-R250 in the same solution for 1.5 hr. The gels were destained with methanol/acetic acid/water (5:7:88, v/v). Bands on sodium dodecyl sulfate gels were scanned with a laser scanning densitometer (LKB Instruments, Inc., Rockville, Md.).

Biochemical analysis. Cytochrome P-450 and/or P-420 contents were estimated by measuring the CO-difference spectra following reduction with dithionite (19). Heme oxygenase activity of hepatic microsomes was determined by the method of Maines and Kappas (20), using hemin as the substrate. The microsomal protein content was quantitated by the procedure of Lowry *et al.* (21), using bovine serum albumin as the standard.

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

Chemicals. Steapsin and hemin, as well as electrophoresis standards, were obtained from Sigma Chemical Company (St. Louis, Mo.). [3,5-^{3}H]δ-aminolevulinic acid and Aquasol were purchased from New England Nuclear Corporation; cadmium chloride and sodium hydrosulfite were obtained from Fisher Scientific Company (Pittsburgh, Pa.); acrylamide and *N,N'*-methylene bisacrylamide were purchased from Eastman Laboratory and Specialty Chemicals (Rochester, N. Y.). All other chemicals were obtained from the J. T. Baker Chemical Company (Phillipsburg, N. H.).

RESULTS

Effect of gender and hypophysectomy on the turnover of hepatic cytochrome P-450. Figure 1 shows the rate of disappearance of radioactive heme associated with microsomal cytochrome P-450 of adult male and female rats (sham-operated). Since rat liver is known to contain at least six different forms of cytochrome P-450 (12–14), the biphasic disappearance profiles suggest the possibility that different forms of cytochrome P-450 might have similar turnover rates. The half-lives of the FP and SP

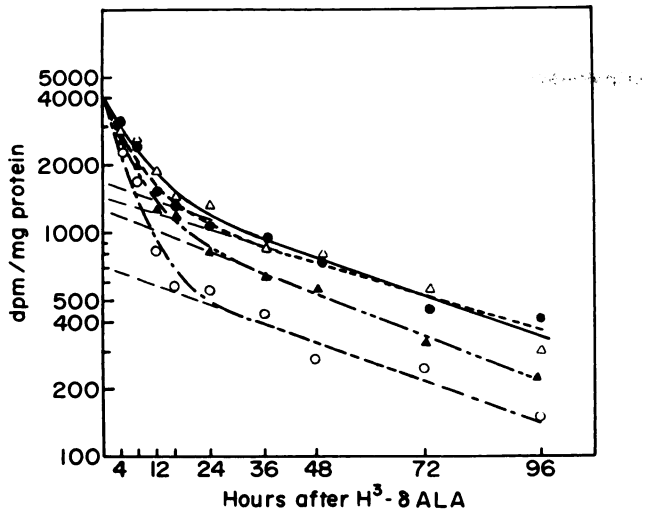

FIG. 1. *Effect of sex and Hx on the disappearance of labeled cytochrome P-450 heme from microsomal CO-binding particles obtained from rat livers*

Male and female rats were hypophysectomized or sham-operated at 50 days of age and received a single i.v. injection of [^{3}H]δ-amininolevulinic acid (*δALA*) (70 μCi/kg body of weight) 30 days after surgery. These animals were killed at different times up to 96 hr following δ-aminolevulinic acid injection. Each point represents the mean of results from four to seven animals. The curves were filled to the data using least-squares regression analysis. Values used for the estimation of the corrected half-life of the FP were obtained by extrapolating the SP to time zero and then subtracting the values of the extrapolated SP from the uncorrected FP portion (total) of the curve (11). Percentages of CO-binding particles represented by the corrected FP and SP hemoprotein (FP/SP component ratio) were obtained by extrapolating the lines to zero time. ▲, Intact male; △, hypophysectomized male; ○, intact female; ●, hypophysectomized female.

hemoproteins were 4.6–5.2 and 40.4–44.5 hr, respectively, in either male or female rats. The total microsomal cytochrome P-450 contents were similar in males and females; however, the ratio of the FP and SP hemoprotein was clearly sex-related (Table 1). The values of the FP/SP ratio were 2.3 and 5.1 for males and females, respectively. Our results are consistent with the findings of Chung (9) and Levin and Ryan (10). Sex differences in hepatic cytochrome P-450 of rats appear to be fully developed by 50 days of age (22). Accordingly, 50-day-old rats were hypophysectomized and the turnover characteristics of hepatic cytochrome P-450 were assessed 30 days later. Data presented in Fig. 1 and Table 1 show that Hx did not alter the half-lives of the FP or SP hemoprotein dramatically in either sex. However, it reduced the values of the FP/SP ratio to 1.1 and 1.8, respectively, for males and females; this is reflected in the preferential increase in the concentration of the SP component of these animals. These data suggest that the sex difference in hepatic cyrochrome P-450 of adult rats is associated with the SP hemoprotein(s) and that these sex differences can be at least partially abolished by postpubertal Hx.

Effect of Cd on the turnover of cytochrome P-450 in male and female rats. Our previous study (6) had shown that postpubertal Hx enhances the Cd responsiveness of hepatic cytochrome P-450 in both male and female rats and abolishes the sexual dimorphism in the response to Cd. Accordingly, we have examined the effect of Cd on the turnover of hepatic cytochrome P-450 in hypophysectomized and sham-operated rats of both sexes to determine whether Cd would preferentially affect the SP hemoprotein. Data presented in Fig. 2 and Table 2 show that Cd treatment increased the FP/SP ratio from 2.3 to 5.3 in male rats; this was apparently due to the selective decrease in the SP fraction (68%) as compared with the FP fraction (31%). In addition, Cd treatment decreased the half-life of the FP component without significantly altering that of the SP component. Table 2 also shows that Hx altered the effect of Cd on the turnover of cytochrome P-450 in male rats. In hypophysectomized male rats, Cd treatment produced no apparent effect on the half-life of the SP component, and the amount of FP hemoproteins was reduced by only 19%. Although the half-life of the SP component was not dramatically altered by Cd, the content of this component was markedly decreased (65% reduction) by treatment of hypophysectomized male rats with Cd.

As shown in Table 3, Cd produced no apparent effect on the total content or turnover of hepatic cytochrome P-450 of adult female rats. In contrast, Cd treatment

TABLE 1

Effect of sex and Hx[a] on the FP and SP components[b] of microsomal CO-binding particles of rat liver

	Cytochrome P-450	Relative amounts[c]		Ratio of FP/SP fractions[c]	$t_{1/2}$[d]	
	(mean ± SD)	FP component	SP component		TP component	SP component
	nmoles/mg protein				*hr*	
Male rats						
Sham-operated	0.64 ± 0.08	0.45	0.19	2.3	5.2	40.4
Hypophysectomized	0.90 ± 0.11	0.47	0.43	1.1	6.0	44.7
% Increase	41	4	126			
Female rats						
Sham-operated	0.55 ± 0.10	0.46	0.09	5.1	4.6	44.5
Hypophysectomized	0.90 ± 0.14	0.58	0.32	1.8	5.0	54.1
% Increase	64	26	256			

[a] Male and female rats were hypophysectomized or sham-operated at 50 days of age and the experiment was initiated 28 days after surgery.

[b] Data were generated from the disappearance of labeled cytochrome P-450 heme from microsomal CO-binding particles obtained from hypophysectomized and sham-operated animals as presented in Fig. 1.

[c] Values were calculated from the ratios of FP/SP hemoprotein fractions.

[d] Computations of the half-lives of hemoprotein fractions and ratios of FP/SP fractions were carried out as described in Fig. 1.

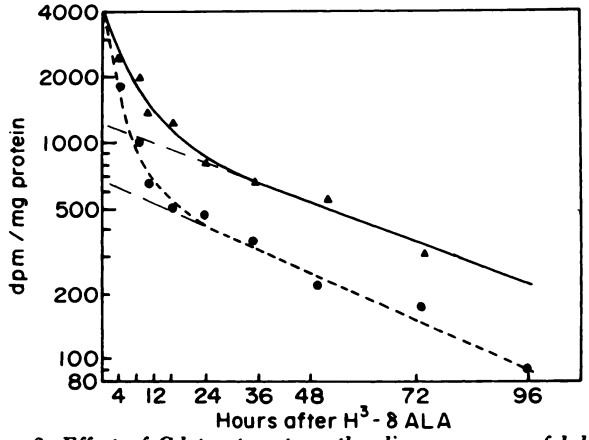

FIG. 2. *Effect of Cd treatment on the disappearance of labeled cytochrome P-450 heme from microsomal CO-binding particles obtained from adult male rat livers*

Male 80-day-old rats (sham-operated) received injections of [^{3}]δ-aminolevulinic acid (*δALA*) (70 μCi/kg, i.v.) 48 hr following the administration of a single dose of $CdCl_2$ (2 mg/kg, i.p.) or an equivalent volume of saline. Computation of various parameters was carried out as stated in Fig. 1. Each point represents the mean of results from three to seven animals. ▲, Control; ●, Cd-treated.

diminished the total cytochrome P-450 content by 24% in hypophysectomized female rats. The decrease was mostly associated with the SP component. The percentage decreases of the SP and FP components over their corresponding control values were 41 and 16, respectively. Nevertheless, the half-lives of these two components were not altered by Cd treatment.

These data demonstrate a clear association between decreases in SP hemoproteins and Cd-mediated decreases in cytochrome P-450 content.

Effect of Cd treatment on the electrophoretic profile of microsomal proteins isolated from liver of sham-operated and hypophysectomized male and female rats. To characterize further the effect of Cd on hepatic cytochrome P-450, we determined the SDS-PAGE profiles of microsomes isolated from livers of sham-operated and hypophysectomized male and female rats. Cd treatment did not change microsomal protein contents per gram of liver in either male or female rats. Examination of the staining intensity of protein bands of control and Cd-treated sham-operated male rats (Fig. 3, Wells *5* and *6*) shows that Cd treatment (2.0 mg/kg) markedly decreased the intensity of at least two protein bands with apparent

TABLE 2

Effect of Cd treatment on FP and SP components of hepatic microsomal CO-binding particles of sham-operated and hypophysectomized male rats

Data were generated from the disappearance of labeled cytochrome P-450 heme from microsomal CO-binding particles derived from hypophysectomized and sham-operated male rats. Male rats were hypophysectomized or sham-operated at 50 days of age and received a single-dose injection of Cd or saline 28 days after surgery. An i.v. dose of [^{3}H]δ-aminolevulinic acid (70 μCi/kg) was given 48 hr following Cd or saline treatment, and the animals were killed 4, 8, 16, 24, 36, 48, 72, and 96 hr later. Three or four animals were used at each time point.

	Cytochrome P-450 (mean ± SD)	Relative amounts[a]		Ratio of FP/SP fractions[a, b]	$t_{1/2}$[a]	
		FP component	SP component		FP component	SP component
	nmoles/mg protein	*hr*				
Sham-operated rats						
Saline	0.64 ± 0.08	0.45	0.19	2.3	5.2	40.4
Cd (2 mg/kg)	0.37 ± 0.08	0.31	0.06	5.3	3.1	44.5
% of decrease	42	31	68			
Hypophysectomized rats						
Saline	0.90 ± 0.11	0.47	0.43	1.1	6.0	44.7
Cd (2 mg/kg)	0.53 ± 0.09	0.38	0.15	2.5	6.1	52.3
% of decrease	41	19	65			

[a] Computations of the half-lives of hemoprotein fractions and ratios of FP/SP fractions were carried out as described in Fig. 1.
[b] Values were calculated from the ratios of FP/SP hemoprotein fractions.

TABLE 3

Effects of Cd treatment on the FP and SP components of hepatic microsomal CO-binding particles of sham-operated and hypophysectomized female rats

Data were generated from the disappearance of labeled cytochrome P-450 heme from microsomal CO-binding particles derived from hypophysectomized and sham-operated female rats. This experiment was carried out as described in Table 2, except that female rats were used.

	Cytochrome P-450 (mean ± SD)	Relative amounts[a]		Ratio of FP/SP fractions[b]	$t_{1/2}$[b]	
		FP component	SP component		FP component	SP component
	nmoles/mg protein				*hr*	
Sham-operated rats						
Saline	0.55 ± 0.10	0.46	0.09	5.1	4.6	44.5
Cd (2 mg/kg)	0.52 ± 0.08	0.49	0.08	6.1	4.4	48.2
% of decrease	—	—	—			
Hypophysectomized rats						
Saline	0.90 ± 0.14	0.58	0.32	1.8	5.0	54.1
Cd (2 mg/kg)	0.68 ± 0.09	0.49	0.19	2.6	4.6	56.6
% of decrease	24	16	41			

[a] Values were calculated from the ratios of FP/SP hemoprotein fractions.
[b] Computations of the half-lives of hemoprotein fractions and ratios of FP/SP fractions were carried out as described in Fig. 1.

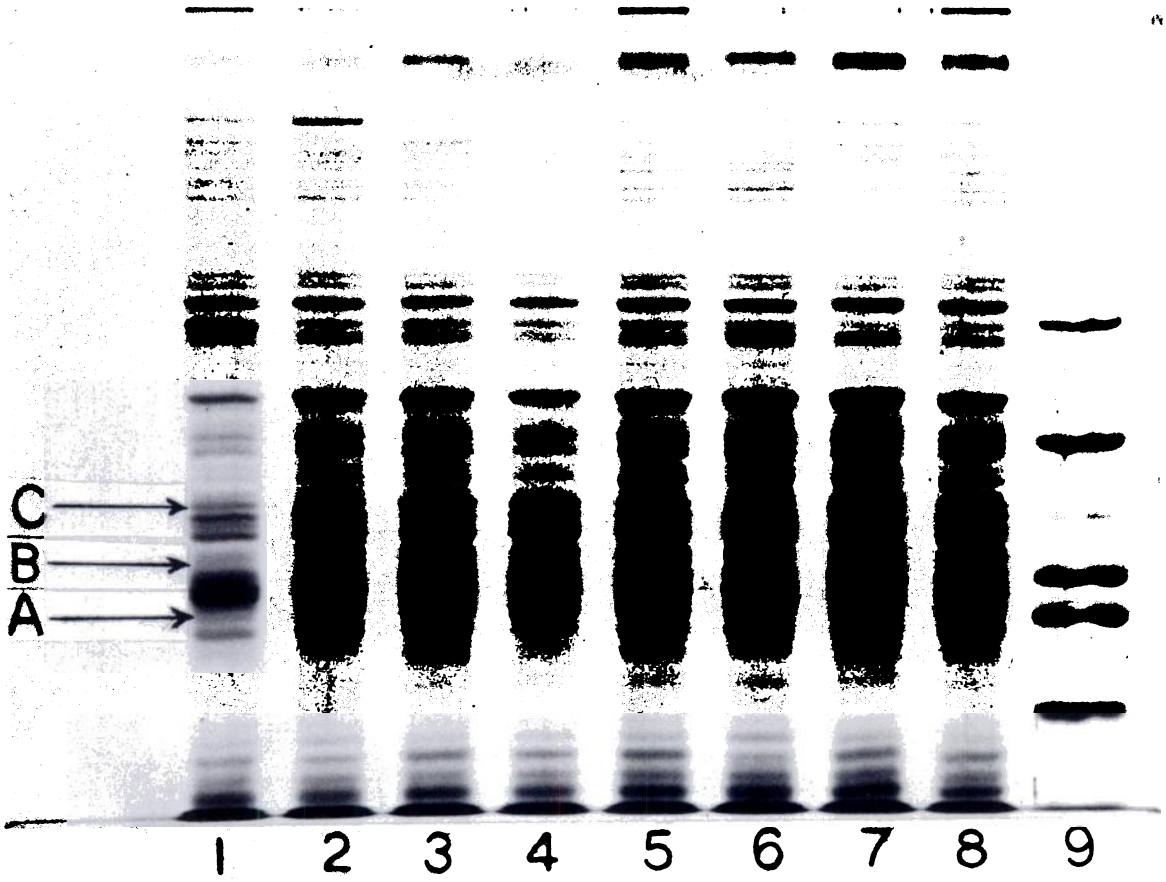

FIG. 3. *SDS-PAGE of rat hepatic microsomes (40 μg)*

Hypophysectomized or sham-operated animals were treated either with Cd (2 mg/kg) or with vehicle only. The *numbered wells* represent the following: *1*, sham-operated females; *2*, sham-operated females + Cd; *3*, hypophysectomized females; *4*, hypophysectomized females + Cd; *5*, sham-operated males; *6*, sham-operated males + Cd; *7*, hypophysectomized males; *8*, hypophysectomized males + Cd; *9*, standards (phosphorylase b, 94,000 mol wt; bovine serum albumin, 68,000 mol wt; catalase, 60,000 mol wt; glutamic dehydrogenase, 53,000 mol wt; fumarase, 49,000 mol wt; and aldolase, 40,000 mol wt).

molecular weights of 54,000 (Band *B*) and 62,000 (Band *C*), and possibly decreased another at 49,000 (Band *A*). The 49,000 mol wt band (Band *A*) is detected as a broad shoulder which is diminished by Cd treatment (Fig. 4). The decrease in staining intensity of these bands was verified by laser scanning densitometry (Fig. 4A). Hx (Well *7*) resulted in increased staining intensity of these three bands relative to controls (Well *5*). Furthermore, Bands *A*, *B*, and *C* from hypophysectomized male rats were decreased by Cd treatment (Well *8*) (Figs. 3 and 4B).

The electrophoretic pattern of microsomal proteins from sham-operated females (Well *1*) was different from that of males (Well *5*). The staining of Bands *A*, *B*, and *C*, in particular, was less intense in females. Furthermore, Cd treatment produced only slight effects on these protein bands (Well *2*) in females (Fig. 4C). Hx of females produced a staining pattern which was similar to that of intact males, especially in the 40,000–70,000 mol wt range. Moreover, Cd treatment of hypophysectomized female rats reduced the degree of staining of Bands *A*, *B*, and *C* (Well *4*) (Fig. 4D).

Effect of gender and Hx on the Cd-induced increase in hepatic microsomal heme oxygenase activity. Hepatic cytochrome P-450 has been considered as a potential substrate for the microsomal heme oxygenase. Accordingly, we examined the effect of Cd treatment on the hepatic heme oxygenase activity of sham-operated and hypophysectomized rats to determine a possible correlation between the induction of heme oxygenase and the Cd-mediated alteration of cytochrome P-450 turnover. Data presented in Table 4 show that the basal activity of heme oxygenase of adult rats exhibited sex differences in that activities in males were higher than in females. Hx resulted in significant decreases in the hepatic heme oxygenase activity of both sexes and decreased their sex-related differences. Cd treatment increased the heme oxygenase activity markedly in sham-operated male rats; a 250%–310% increase over control activities was observed at 24, 48, and 72 hr following treatment. In contrast to males, treatment of females with Cd resulted in only a 100%–123% increase in heme oxygenase activity at 23 and 48 hr following treatment, and no alteration of enzyme activity could be detected at 72 hr. The data in Table 4 also show the effect of Hx on the Cd-induced increase in hepatic heme oxygenase activity. Although Cd responsiveness was decreased in hypophysectomized male rats, a 160%–240% increase in enzyme activity was

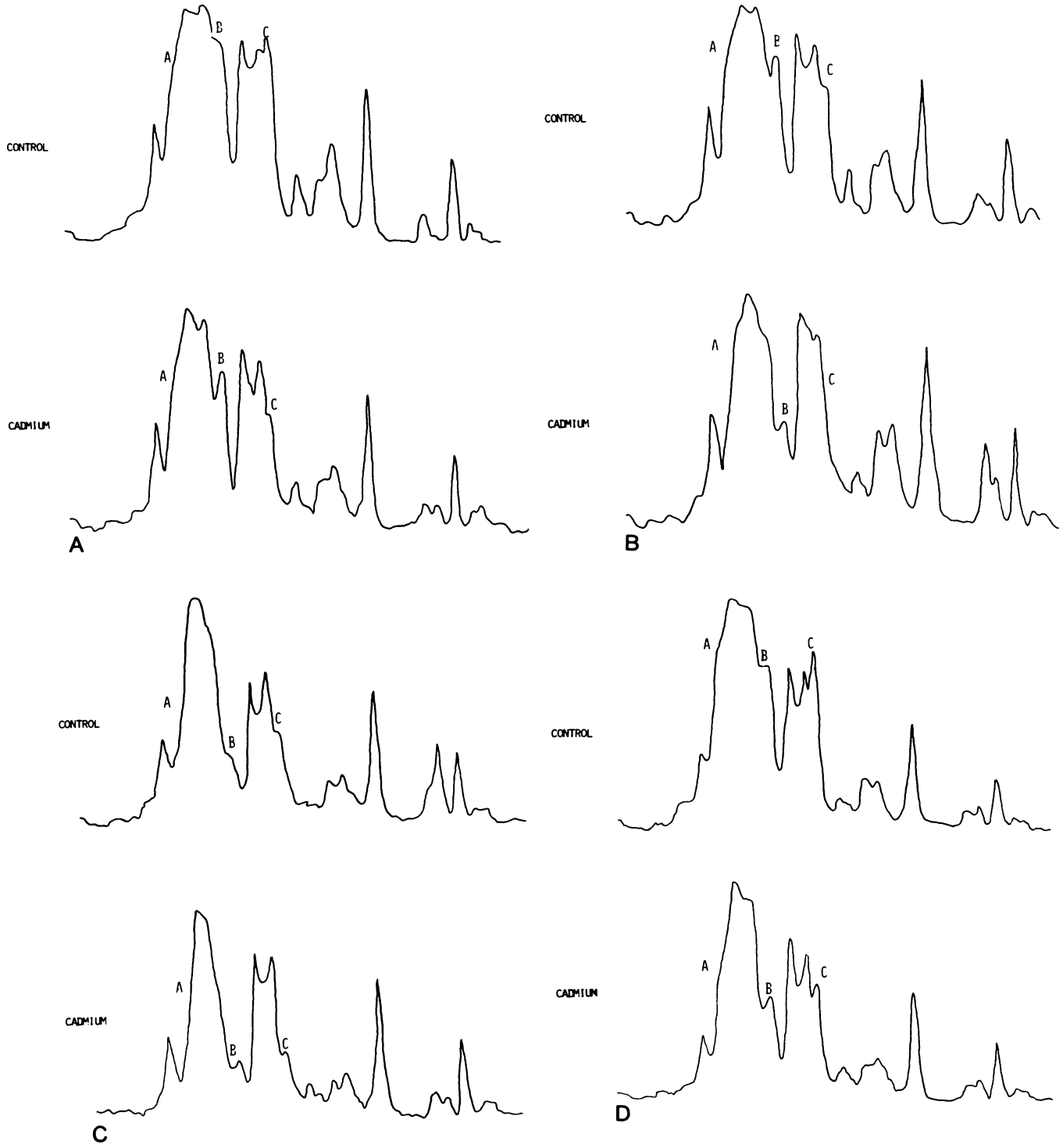

FIG. 4. *Profiles of hepatic microsomal proteins (Bands A, B, and C) derived by laser scanning densitometry*

A, Sham-operated males and Cd-treated sham-operated males; B, hypophysectomized males and hypophysectomized males treated with Cd; C, sham-operated females and Cd-treated sham-operated females; D, hypophysectomized females, and hypophysectomized females treated with Cd.

still detected in this group of animals. This Cd-induced heme oxygenase activity was only one-third that of intact Cd-treated male rats (sham-operated) and was similar to that detected in Cd-treated females. However, Hx of females did not alter Cd responsiveness of heme oxygenase. The magnitude as well as the time course of the increase in enzyme activity was similar in sham-operated and hypophysectomized females. These data reveal that

TABLE 4

Effect of Cd treatment on the microsomal heme oxygenase activity of sham-operated and hypophysectomized rats

Rats were sham-operated or hypophysectomized at 50 days of age and received a single i.p. dose of saline or $CdCl_2$ (2 mg/kg) 27 days after surgery.

	Heme oxygenase[a]			
	Saline-treated controls	Time following Cd treatment		
		24 hr	48 hr	72 hr
	pmoles bilirubin formed/min/mg protein			
Male rats				
Sham-operated	60 ± 9	250 ± 50[b] (216)	235 ± 45[b] (291)	210 ± 50[b] (250)
Hypophysectomized	25 ± 5	85 ± 10[b] (240)	75 ± 9[b] (200)	65 ± 10[b] (160)
Female rats				
Sham-operated	40 ± 7	89 ± 10[b] (123)	80 ± 11[b] (100)	42 ± 5
Hypophysectomized	20 ± 4	52 ± 6[b] (160)	35 ± 5[b] (75)	20 ± 5

[a] Values are the means ± standard deviation of 16 rats in the control group and 6–8 rats in the Cd-treated group. Values in parentheses represent the percentage increase over control activities.

[b] Significantly different from saline-treated controls ($p < 0.05$).

Hx abolished sex differences in the basal activities and slightly decreased Cd inducibility of hepatic heme oxygenase.

DISCUSSION

Recent studies have shown that acute Cd treatment reduces the specific content of hepatic cytochrome P-450 of adult male but not female rats (1, 6) and that postpubertal Hx increases Cd responsiveness in both sexes and significantly decreases sex-related differences (6). The findings of the present study suggest that the effect of gender and Hx on Cd responsiveness of hepatic cytochrome P-450 is associated with changes in concentration of the SP hemoproteins. The hepatic cytochrome P-450 contents of adult male and female rats were similar; however, concentrations of the SP component were higher in males than in females (Table 1). Moreover, the increase in hepatic cytochrome P-450 content detected in male and female rats following Hx was associated with concomitant elevation of the SP hemoprotein fraction (Table 1). Therefore, the degree of Cd responsiveness of hepatic cytochrome P-450 appears to be associated with the abundance of Cd-sensitive cytochrome P-450(s). However, turnover studies do not discriminate among forms of cytochrome P-450 because the resolution of the FP- and SP-labeled hemoprotein fractions depends only on the rate of disappearance of the heme moieties associated with multiple forms of cytochrome P-450. Therefore, it is conceivable that the SP component could be made up of several cytochrome P-450s with similar half-lives. In support of this view, the data on the SDS-PAGE of hepatic microsomal preparations (Fig. 3) indicate that there were several proteins that exhibited sex differences and that these protein bands were sensitive to the effects of Hx and Cd treatment. Analysis of the electrophoretic profiles of adult rat (sham-operated) reveal that the staining of at least three protein bands (Bands *A*, *B*, and *C*) was more intense in males than in females, and the staining intensities of males were significantly decreased by Cd treatment while only slight reductions were evident in females. These findings are similar to the effects of Cd on the SP hemoprotein(s) (Tables 2 and 3). Furthermore, the increases in the staining intensity of Bands *A*, *B*, and *C* in male and female rats following Hx correspond to similar increases in the SP hemoprotein contents of these animals (Table 1). In addition, decreases in protein Bands *A*, *B*, and *C* of hypophysectomized rats of either sex following Cd treatment correlate with the Cd-mediated decreases in SP component levels of these animals.

Although selective effects of Cd on rat hepatic cytochrome P-450 are evident, the mechanism of Cd-mediated decreases of hepatic cytochrome P-450 is still not well understood. Data from our turnover studies suggest that the Cd-mediated depression of the SP hemoprotein fraction in adult male rats was due to decreased synthesis rather than increased degradation, since the half-life of this pulse-labeled hemoprotein fraction was not reduced by Cd treatment (Table 2). Our finding is similar to that reported by Means and Schnell (8), but differs from that of Krasny and Holbrook (2), who have reported a decrease in the half-life of the SP component following Cd treatment. In view of the effect of Cd on hemoprotein synthesis, the selective inhibitory effect of Cd on different cytochrome isozymes may be due to preferential repression of the synthesis of these "Cd-sensitive cytochrome P-450s." Moreover, the pituitary glands appear to play a modulating role in the Cd-induced decrease in hemoprotein synthesis, as indicated by the detectable effects of Cd on SP hemoprotein levels in hypophysectomized but not intact females (Table 3). It remains to be determined whether the pituitary-dependent Cd-antagonizing factor postulated in our previous study (6) would suppress the synthesis of the "Cd-sensitive cytochrome P-450." This would provide a mechanism for the effect of gender and Hx on the Cd-induced decrease in cytochrome P-450 synthesis.

Evaluation of microsomal protein profiles by SDS-PAGE revealed some interesting findings important to understanding the nature of pituitary regulation of hepatic protein synthesis. The pituitary is known to play a dominant role in the regulation of sex differences in a wide variety of live proteins (9, 23–25). It has been postulated that the pituitary, under hypothalamic influences, releases a feminizing factor which is critical to the expression of hepatic sex differences (26). The release of the pituitary feminizing factor is prevented in males because neonatal androgens apparently program for the synthesis of a hypothalamic inhibiting factor which prevents release of the pituitary feminizing factor (26). Our data support the concept of a feminizing factor(s) in that liver microsomal protein profiles of hypophysectomized females are similar to intact male profiles (Figs. 3 and 4). Hypophysectomy was especially effective in abolishing sex differences in microsomal proteins detected in the 40,000–70,000 mol wt range. Therefore, it appears that pituitary feminizing factors are responsible for many of the sex differences in hepatic microsomal enzymes.

Although we have focused the current studies on Cd

effects on SP hemoprotein(s), Cd also increased the degradation of the FP hemoprotein component in a sex- and pituitary-dependent manner, as indicated by the Cd-mediated decrease in the half-life of this hemoprotein component in adult males. This effect is not observed in females or in hypophysectomized animals of either sex (Tables 2 and 3). Since heme oxygenase constitutes the rate-limiting step in the degradation of heme and the heme moiety of cytochrome P-450 is a potential substrate of heme oxygenase (27), the effect of Cd on hepatic heme oxygenase must be evaluated as a factor in the sex- and pituitary-dependent effects of Cd on cytochrome P-450 degradation. However, the cause/effect relationship between induction of heme oxygenase and degradation of cytochrome P-450 is not well defined. It is thought that the increase in heme oxygenase activity following exposure to various metals (e.g., cobalt) is responsible for the decrease in hepatic cytochrome P-450 concentration (20). Alternatively, it has been suggested that Cd exposure causes an increase in tissue concentration of free heme or free hemoproteins which could subsequently induce heme oxygenase (27). It seems clear from the data presented in Tables 3 and 4 that the sex-related effects of Cd on total hepatic cytochrome P-450 concentration are not associated with changes in heme oxygenase activity. For example, treatment of female rats with Cd resulted in a 2-fold increase in heme oxygenase activity without altering the degradation or the content of cytochrome P-450. This finding implies that induction of hepatic heme oxygenase does not necessarily result in an increase in the rate of cytochrome P-450 degradation or a decrease in hemoprotein contents of the liver. It also provides evidence that cytochrome P-450 is not a good substrate for heme oxygenase, which is consistent with the report that cytochrome P-450 must be converted to cytochrome P-420 prior to degradation by heme oxygenase (28). Since Hx enhances the effect of Cd on cytochrome P-450 without significant effects on the induction of heme oxygenase by Cd, further evidence is provided that heme oxygenase activity does not play an important role in the sex- and pituitary-related effects of Cd on cytochrome P-450.

Our results demonstrate that the selective effects of Cd on hepatic cytochrome P-450 of adult male rats appear to reflect the concentrations of Cd-sensitive forms of cytochrome P-450 in hepatic microsomes. Moreover, the selective actions of Cd suggest heterogeneity in the regulatory processes controlling the levels of different cytochrome P-450 isozymes. Although we have not characterized the mechanism(s) underlying the regulation of the Cd-sensitive forms of cytochrome P-450, the sensitivity of the monooxygenase system to Cd may be used as a criterion or a probe (in conjunction with the effects of gender and Hx) for studying these forms of cytochrome P-450.

REFERENCES

1. Schnell, R. C., J. R. Means, S. A. Roberts, and D. H. Pence. Studies on cadmium-induced inhibition of hepatic microsomal drug biotransformation in the rat. *Environ. Health Perspect.* **28**:273–279 (1979).
2. Krasny, H. R., and D. J. Holbrook, Jr. Effects of cadmium on microsomal hemoproteins and heme oxygenase in rat liver. *Mol. Pharmacol.* **13**:759–765 (1977).
3. Hadley, W. M., T. S. Miya, and W. F. Bousquet. Cadmium inhibition of hepatic drug metabolism in the rat. *Toxicol. Appl. Pharmacol.* **28**:284–291 (1974).
4. Pence, D. H., T. S. Miya, and R. C. Schnell. Cadmium alteration of hexobarbital action: sex-related differences in the rat. *Toxicol. Appl. Pharmacol.* **39**:89–96 (1977).
5. Lui, E. M. K., and G. W. Lucier. Neonatal feminization of hepatic monooxygenase in adult male rats: altered sexual dimorphic response to cadmium. *J. Pharmacol. Exp. Ther.* **212**:211–216 (1980).
6. Lui, E. M. K., and G. W. Lucier. Hypophyseal regulation of cadmium-induced depression of the hepatic monooxygenase system in the rat. *Mol. Pharmacol.* **20**:165–172 (1981).
7. Means, J. R., G. P. Carlson, and R. C. Schnell. Studies on the mechanism of cadmium-induced inhibition of the hepatic microsomal monooxygenase of the male rat. *Toxicol. Appl. Pharmacol.* **48**:293–304 (1979).
8. Means, J. R., and R. C. Schnell. Cadmium-induced alteration of the microsomal monooxygenase system in male rat liver: effects on hemoprotein turnover and phospholipid content. *Toxicol. Lett.* **3**:177–184 (1979).
9. Chung, L. W. K. Characteristics of neonatal androgen-induced imprinting of rat hepatic microsomal monooxygenases. *Biochem. Pharmacol.* **26**:1979–1984 (1977).
10. Levin, W., and D. Ryan. Age and sex differences in the turnover of rat liver cytochrome P-450: the role of neonatal imprinting, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (P. L. Morselli, S. Garattini, and F. Sereni, eds.). Raven Press, New York, 265–275 (1975).
11. Levin, W., and R. Kuntzman. Biphasic decreases of radioactive hemoprotein from liver microsomal CO-binding particles: effect of 3-methylcholanthrene. *J. Biol. Chem.* **244**:3671–3676 (1969).
12. Thomas, P. E., A. Y. H. Lu, D. Ryan, S. B. West, J. Kawalek, and W. Levin. Multiple forms of rat liver cytochrome P-450. *J. Biol. Chem.* **251**:1385–1391 (1976).
13. Ryan, D. E., P. E. Thomas, D. Korzeniowski, and W. Levin. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, and 3-methylcholanthrene. *J. Biol. Chem.* **254**:1365–1374 (1979).
14. Guengerich, F. P. Separation and purification of multiple forms of microsomal cytochrome P-450. *J. Biol. Chem.* **252**:3970–3979 (1977).
15. McIntosh, P. R., A. C. Peacock, and H. V. Gelboin. The induction of liver microsomal proteins by 3-methylcholanthrene in rats of different age, sex and strain. *Biochim. Biophys. Acta* **627**:290–300 (1980).
16. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Regulation of three forms of cytochrome P-450 and epoxide hydrolase in rat liver microsomes. *J. Biol. Chem.* **256**:1044–1052 (1981).
17. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature (Lond.)* **227**:680–685 (1970).
18. Weber, K., and M. Osborn. The reliability of molecular weight determinations by dodecyl-sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406–4412 (1969).
19. Omura, T., and R. Sato. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**:2370–2378 (1965).
20. Maines, M. D., and A. Kappas. Cobalt stimulation of heme degradation in the liver. *J. Biol. Chem.* **250**:4171–4177 (1975).
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
22. El Defrawy El Masry, S., G. M. Cohen, and G. J. Mannering. Sex-dependent differences in drug metabolism in the rat. I. Temporal changes in the microsomal drug-metabolizing system of the liver during sexual maturation. *Drug Metab. Dispos.* **2**:267–278 (1974).
23. Kramer, R. E., J. W. Greiner, R. C. Rumbaugh, T. D. Sweeney, and H. D. Colby. Requirement of the pituitary gland for gonadal hormone effects on hepatic drug metabolism in rats. *J. Pharmacol. Exp. Ther.* **208**:19–23 (1979).
24. Skett, P., and J. A. Gustafsson. Imprinting of enzyme systems of xenobiotic and steroid metabolism, in *Reviews in Biochemical Toxicology* (E. Hodgson, J. Bend, and R. Philpot, eds.). Elsevier/North-Holland, 27–52 (1979).
25. Rumbaugh, R. C., and H. D. Colby. Is growth hormone the pituitary feminizing factor mediating the actions of estradiol on hepatic drug and steroid metabolism? *Endocrinology* **107**:719–724 (1980).
26. Gustafsson, J. A., A. Larson, P. Skett, and A. Stenberg. Influence of a novel hypophyseal factor on steroid metabolism in cultured hepatoma cells. *Proc. Natl. Acad. Sci. U. S. A.* **72**:4986–4990 (1975).
27. Maines, M. D., M. W. Anders, and U. Muller-Eberhard. Studies on heme transfer from microsomal hemoproteins to heme-binding plasma proteins. *Mol. Pharmacol.* **10**:204–213 (1974).
28. Maines, M. D. Mechanism of degradation of endogenous heme and cytochrome P-450 by heme oxygenase, in *Microsomes and Drug Oxidations* (V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, eds.). Pergamon Press, New York, 543 (1977).

Send reprint requests to: Dr. George W. Lucier, Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, N. C. 27709.