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EFFECTS OF INHIBITORS OF RNA AND PROTEIN SYNTHESIS ON HEPATIC MICROSOMAL CYTOCHROME P-450 CONCENTRATION IN FASTED AND FED RATS

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Abstract—A number of sex-related differences in the metabolism of drugs, steroids and xenobiotics have been reported in studies on rats. Generally, male rats tend to metabolize these compounds more efficiently than females. In the studies presented here, male and female rats were fasted for 24–48 hr, and the effects of fasting on total hepatic microsomal cytochrome P-450 were examined. Hepatic cytochrome P-450, as determined by CO difference spectra, was increased significantly as a percentage of control in microsomes from fasted female rats when compared to fasted male rats. Cytochrome P-450 concentration increased from 0.57 ± 0.07 nmole/mg protein to 0.99 ± 0.08 nmole/mg protein following a 24-hr fast. In male rats, cytochrome P-450 levels were essentially unaffected by the 24-hr fast. Cytochrome b_5 concentration was not altered by fasting. When female rats were fasted for 24 hr and refed, cytochrome P-450 levels were not significantly different from cytochrome P-450 levels in continuously fed animals. Treatment of fasted female rats with the protein synthesis inhibitor ethionine, or the RNA synthesis inhibitor actinomycin D, prevented the induction of cytochrome P-450 in the fasting animal. Cytochrome P-450 concentration in fed animals was not affected significantly by either inhibitor. Induction of cytochrome P-450 by phenobarbital (PB) and 3-methylcholanthrene (MC) under fed and fasted conditions was also investigated in male and female rats. Xenobiotic-induced cytochrome P-450 concentration was significantly higher in fasted female hepatic microsomes when compared to microsomes from fed female rats. Fasting did not significantly affect xenobiotic-induced cytochrome P-450 in male rats. Our results suggest that fasting in female rats results in an increase in cytochrome P-450 which is dependent upon synthesis of RNA and protein.

The term cytochrome P-450 refers to a group of isozymes which catalyze the insertion of an oxygen atom from molecular oxygen into a variety of compounds such as drugs, steroids and carcinogens [1, 2]. Due to the multiple forms of cytochrome P-450, these isozymes are capable of metabolizing a wide variety of structurally unrelated compounds [3, 4]. The various forms of cytochrome P-450 are highly inducible and show some degree of substrate specificity [3, 4]. Compounds such as phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, and isosafrole have been shown to induce distinct forms of cytochrome P-450 [5–7].

Sex differences in hepatic metabolism of a number of different drugs, steroids and xenobiotics have been well documented [8–10]. Most cytochrome P-450-mediated reactions are more effective in the male rat. However, exceptions have been reported [9]. The concentration of cytochrome P-450 is higher in hepatic microsomes prepared from male rats than in those from female rats. These sex differences in drug and steroid metabolism in the rat have been attributed primarily to the action of gonadal steroids, mainly androgens [11]. Sex differences in the pattern of cytochrome P-450 in rat liver microsomes have also been observed [12].

Starvation of female rats has been shown to enhance the activity of most drug-metabolizing enzymes in liver microsomes, whereas starvation of male rats results in impairment of the activity of sex-dependent, drug-metabolizing enzymes [13, 14]. Studies by Bock *et al.* [15] showed that fasting for 24 hr results in a decrease in cytochrome P-450 in male rat liver microsomes. A recent study by Mohn *et al.* [16], using gel electrophoresis, showed that starvation results in a sex-dependent increase in hepatic microsomal cytochrome P-450 in female mice.

In order to better understand how fasting influences cytochrome P-450-mediated reactions in male and female rat liver microsomes, this study was carried out to determine the effects of both RNA and protein synthesis inhibitors on fed and fasting cytochrome P-450 concentrations in hepatic microsomes.

MATERIALS AND METHODS

Animals and treatment. Male and female Sprague–Dawley rats weighing 120–140 g were obtained from the Holtzman Co. (Madison, WI). All animals were kept under controlled conditions (22°, lights on 6:00 a.m. to 8:00 p.m.). Animals were either fed *ad lib.* or fasted for the indicated time under each experimental protocol. Animals were fed the standard Purina Rat Chow diet. Fasting rats were kept in metabolic cages to ensure proper fasting by pre-

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venting coprophagy. DL-Ethionine was administered i.p. in a single dose of 500 mg/kg body weight. DL-Methionine was given i.p. in a single dose of 460 mg/kg body weight. Actinomycin D in 10% ethanol was given, i.p., in a single dose, at 1 mg/kg body weight. 3-Methylcholanthrene in corn oil was given in a single i.p. injection of a dose of 40 mg/kg body weight. All other drugs were dissolved in 0.9% NaCl (w/v). In each experimental protocol, control animals received appropriate injection vehicles.

Chemicals. NADH, phenobarbital, DL-ethionine, DL-methionine and actinomycin D were obtained from the Sigma Chemical Co. (St. Louis, MO). 3-Methylcholanthrene was purchased from ICN Pharmaceuticals Inc., Life Science Group (Plainview, NY). All other reagents and chemicals employed were of analytical grade.

Isolation of microsomes. In all experiments, livers were perfused *in situ* with 100 ml of cold 0.25 M sucrose. Livers were removed, blotted and weighed. After weighing, livers were minced with scissors and homogenized in a Dounce homogenizer at a 1:4 volume ratio of minced liver to 0.25 M sucrose. The homogenate was centrifuged at 9000 *g* for 20 min. The resulting supernatant fraction was then centrifuged at 105,000 *g* for 1 hr. The microsomal pellets were immediately washed with cold 1.15% (w/v) KCl and recentrifuged at 105,000 *g* for 1 hr. The resulting microsomal pellet was resuspended in 0.25 M sucrose to a protein concentration of 20 mg/ml.

Enzymic assays. Total cytochrome P-450 was assayed according to the procedure of Omura and Sato (using a difference spectral extinction coefficient of 91 mM^{-1} cm^{-1}) for the CO-liganded, reduced hemoprotein [17]. For cytochrome b_5 determinations, 60 μM NADH was added to the sample curvette, and the difference in absorbance 424 nm versus 409 nm was recorded [81]. Protein was determined by the method of Lowry *et al.* [19]. All spectrophotometric assays were done using a Perkin–Elmer double beam, dual wavelength scanning spectrophotometer, model 557. Analysis of variance was employed for statistical analyses. Values are expressed as the mean ± S.D. P values greater than 0.05 were not considered statistically significant.

RESULTS

Effects of fasting. Figure 1 shows the effects of fasting on hepatic microsomal cytochrome P-450 in male and female rats. After 24 and 48 hr fasting, cytochrome P-450 concentration was significantly higher ($P < 0.01$) in fasted female liver microsomes than in fed female rat liver microsomes. However, cytochrome P-450 levels in male rat liver microsomes were not affected significantly by the 24 hr fast. The amount of microsomal protein per gram of liver was virtually the same in male and female rats and did not change markedly over the 24 hr fast. Thus, the observed differences between male and female rat liver microsomal cytochrome P-450 concentration cannot be attributed to changes in the concentration of microsomal protein.

When female rats were fasted for 24 hr and then refed for 48 hr prior to being killed, their cytochrome P-450 levels were reduced to essentially the same levels as those in rats which were fed continuously (Fig. 1). Results of cytochrome b_5 determinations (data not presented) showed that cytochrome b_5 concentration was not affected by fasting in male or female rats.

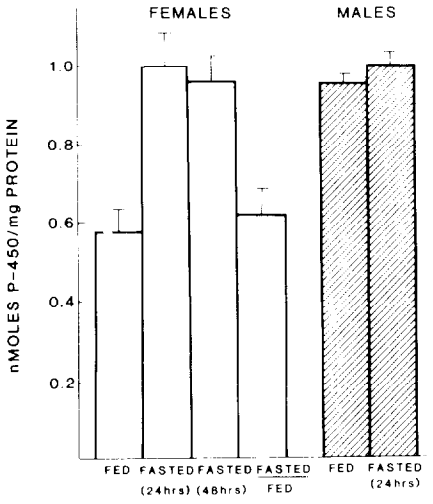

Fig. 1. Female and male rats were either fed or fasted for the indicated times. For fasting and re-feeding experiments, animals were fasted for 24 hr, then fed for 48 hr and killed. Total P-450 was determined according to the procedure of Omura and Sato [17]. Vertical bars represent the mean ± S.D. of four or more separate experiments.

Effects of inhibitor of protein synthesis. To determine whether the increase in cytochrome P-450 concentration was due to increased protein synthesis, the following experiments were done. Female rats, fed and fasted, were given a single i.p. injection of DL-ethionine (500 mg/kg body wt) [20]. Animals were injected at 8:00 a.m. and killed 24 hr later. The data in Table 1 show the effects of DL-ethionine on cytochrome P-450 concentration in microsomes isolated from fed and fasted female rats. Twenty-four hour fasting resulted in an approximate 2-fold increase in hepatic microsomal cytochrome P-450. Cytochrome b_5 concentration was not affected by the fasting. When fasted animals were treated with DL-ethionine, microsomal cytochrome P-450 concentration was not significantly different from fed animals treated with DL-ethionine. Although DL-ethionine treatment resulted in a marked decrease in fasting cytochrome P-450 concentration, total cytochrome P-450 content in fed animals was essentially unaffected by the DL-ethionine treatment. Treatment of fasted female rats concomitantly with DL-ethionine and DL-methionine (460 mg/kg body wt) essentially reversed the inhibitory effect of DL-ethionine on fasting-induced increases in hepatic microsomal cytochrome P-450. Data in Table 1 also shows that DL-methionine and ethionine, when given in combination, had no significant effect on cytochrome P-450 concentration in fed animals.

Effect of RNA synthesis inhibitor. The data in Table 2 show the effects of the RNA synthesis inhibitor actinomycin D on cytochrome P-450 con-

Table 1. Effects of DL-ethionine on hepatic microsomal cytochrome b_5 and cytochrome P-450 in fed and fasted female rats*

Nutritional state	Treatment		Cytochrome b_5 (nmoles/mg protein)	Cytochrome P-450 (nmoles/mg protein)
Fed	None	(5)	0.45 ± 0.02	0.57 ± 0.10
Fed	Ethionine	(5)	0.44 ± 0.03	0.54 ± 0.07
Fasted	None	(5)	0.47 ± 0.04	0.97 ± 0.10†
Fasted	Ethionine	(5)	0.46 ± 0.06	0.66 ± 0.02
Fed	Methionine + ethionine	(5)	0.47 ± 0.04	0.57 ± 0.02
Fasted	Methionine + ethionine	(5)	0.45 ± 0.05	0.85 ± 0.06†

* Female rats were given i.p. injections of DL-ethionine (500 mg/kg body wt) or DL-methionine (460 mg/kg body wt). Animals were either fed or fasted for 24 hr and then killed. Values represent the mean ± S.D. Numbers in parentheses show the number of experiments.

† Significant by different from control, $P < 0.01$.

centration in fed and fasted female rats. Actinomycin D treatment did not affect markedly cytochrome P-450 concentration in microsomes isolated from fed animals. Fasting cytochrome P-450 was increased significantly above control levels. However, fasting cytochrome P-450 concentration was not significantly different from control (fed) when fasting animals were treated with actinomycin D. Again, cytochrome b_5 concentration was not affected by the actinomycin D treatment.

Effect of fasting on xenobiotic-induced cytochrome P-450. Both phenobarbital (PB) and 3-methylcholanthrene (MC) are potent inducers of isozymes of cytochrome P-450 [5, 6]. The data in Table 3 show the effects of fasting on the induction of cytochrome P-450 in liver microsomes from male and female rats. As can be seen in Table 3, treatment of both male and female rats with PB or MC resulted in the characteristic increase in microsomal cytochrome P-450. However, during the 24 hr fast, the increase in cytochrome P-450 following PB and MC treatment was greater as a percentage of control in fasted female rats compared to fasted male rats, resulting in approximately equal induced levels. Cytochrome b_5 concentration was essentially unchanged under all experimental conditions tested.

DISCUSSION

In these studies, fasting resulted in a significant increase in hepatic microsomal cytochrome P-450 in female rats, whereas the level in male rat liver microsomes was not affected (Fig. 1). In agreement with Kato and Gillette [13], we also found that the amount of microsomal protein per gram of liver did not change markedly during the fasting period in male or female rats. Moreover, the specific concentration of cytochrome b_5 was not affected significantly by fasting in male or female rats. Thus, the increase in cytochrome P-450 during fasting in the female could not be attributed to changes in the concentration of microsomal protein. Administration of the protein synthesis inhibitor DL-ethionine prevented the increase in cytochrome P-450 concentration in fasted female rat liver microsomes. Cytochrome P-450 content in fed rats was not affected significantly by DL-ethionine treatment. Therefore, the observed inhibition of cytochrome P-450 increase by DL-ethionine during fasting was not due to some toxic effect of this compound. The inhibitory effect of ethionine could be readily reversed by concomitant administration of methionine (Table 1). This would tend to support the involvement of

Table 2. Effects of actinomycin D on hepatic microsomal cytochrome b_5 and cytochrome P-450 in fed and fasted female rats*

Nutritional state	Treatment		Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)
Fed	None	(5)	0.45 ± 0.02	0.57 ± 0.10
Fed	Actinomycin D	(5)	0.47 ± 0.04	0.58 ± 0.05
Fasted	None	(5)	0.47 ± 0.04	0.97 ± 0.10†
Fasted	Actinomycin D	(5)	0.49 ± 0.03	0.67 ± 0.03

* Female rats were given a single i.p. injection of actinomycin D (1 mg/kg body wt). Animals were then fed *ad lib.* or fasted for 24 hr and killed. Values represent the mean ± S.D. Numbers in parentheses show the number of experiments.

† Significant by different from control, $P < 0.01$.

Table 3. Effects of fasting on xenobiotic induction of hepatic microsomal cytochrome P-450 in male and female rats*

Nutritional state	Sex	Xenobiotic	Cytochrome P-450 (nmoles/mg protein)	% of Fed
Fed	F	None (5)	0.57 ± 0.07	
Fasted	F	None (5)	0.99 ± 0.08†	173
Fed	M	None (5)	0.95 ± 0.02	
Fasted	M	None (5)	1.02 ± 0.05	105
Fed	F	PB (5)	1.20 ± 0.23†	210
Fed	M	PB (5)	1.78 ± 0.03†	187
Fasted	F	PB (5)	1.83 ± 0.35†	320
Fasted	M	PB (5)	1.84 ± 0.08†	194
Fed	F	MC (5)	0.97 ± 0.02†	170
Fed	M	MC (5)	1.44 ± 0.16†	152
Fasted	F	MC (5)	1.31 ± 0.33‡	230
Fasted	M	MC (5)	1.59 ± 0.17†	165

* Male and female rats were given a single i.p. injection of phenobarbital (PB) (80 mg/kg body wt) or 3-methylcholanthrene (MC) (40 mg/kg body wt) and killed 24 hr later. Control animals received injection vehicle only. Values represent the mean ± S.D. Numbers in parentheses show the number of experiments.

†,‡ Significantly different from respective fed or fasted controls: †$P < 0.01$, and ‡$P < 0.05$.

ethionine as an inhibitor of protein synthesis [20]. Administration of actinomycin D, which has been shown to inhibit RNA synthesis [21], also prevented the increase in total cytochrome P-450 concentration in fasted animals. Cytochrome P-450 concentration in fed animals was not affected significantly by the actinomycin D treatment. These data strongly suggest that the increase in total hepatic cytochrome P-450 concentration during fasting involved the synthesis of RNA and protein. Our studies also show that, after fasting and refeeding, total cytochrome P-450 concentration was essentially the same as in animals fed *ad lib.* (Fig. 1). These data show that the increase in cytochrome P-450 induced by fasting was readily reversible by re-feeding. Conceivably, refeeding may result in an increase in an isozyme or isozymes with a short $T_{1/2}$. A recent study has shown that half-lives of cytochrome P-450 isozymes range from 12 to 50 hr [22]. These time intervals are sufficient to account for the observed increases and decreases in P-450 during fasting and re-feeding.

Previous studies by Kato and Gillette [13] showed that fasting for 72 hr results in an increase in some drug-metabolizing enzymes in female rats while decreasing these enzymes in the male rat. It was suggested that starvation may evoke its impairing effects in male rats by interfering with the stimulatory effects of androgenic steroids. It is possible that the stimulation of many of the drug-metabolizing enzymic activities in hepatic microsomes from fasting female rats may be due to increased synthesis of cytochrome P-450(s). Although total cytochrome P-450 concentration increased approximately 2-fold in fasted female rat liver microsomes, the actual increase in one or several of the isozymes of cytochrome P-450 might have been much greater. Both phenobarbital and 3-methylcholanthrene have been shown to be potent inducers of cytochrome P-450 isozymes [5, 6]. The data in Table 3 show that these compounds induced cytochrome P-450 in both male and female rat liver microsomes. Previous studies have shown that during the induction process cytochrome P-450 is increased to a higher level in fasting than in fed rats [23]. Our data are consistent with these findings. Additionally, our studies show that the percent increase in phenobarbital-induced cytochrome P-450 in microsomes from fasted female rats was higher than that in male rat liver (Table 3).

Recently, Gustafsson proposed a new hormonal system for the control of steroid and drug metabolism [24]. This proposal suggested that the hypothalamo-pituitary axis played a pivotal role in determining sex differences in steroid and drug metabolism in rats. Sex differences in the isozyme pattern of cytochrome P-450 in rat liver microsomes have been reported [12, 25]. An interesting speculation would be that fasting in female rats affects these sex-related cytochrome P-450 isozymes, resulting in an increase in their rates of synthesis. Studies are presently underway to ascertain which cytochrome P-450 isozyme(s) is(are) induced upon fasting and what their roles in steroid and xenobiotic metabolism are.

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