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ALTERATIONS IN RAT HEPATIC DRUG METABOLISM DURING PREGNANCY AND LACTATION

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Abstract—The hepatic microsomal drug metabolism during pregnancy and lactation was studied. Four days post partum, the concentrations of cytochrome P450 and cytochrome b5 were reduced by 50% when compared with pregnant rats, at day 10 of gestation. Within this time period the N-demethylation of aminopyrine, the rate of aldrin epoxidation and the N-demethylation of demethylnitrosamine was reduced by 53, 74 and 21%, respectively. However, the rates of ethoxyresorufin-O-deethylation did not differ amongst both groups and the deethylation of 4-nitroanisole and the 4-hydroxylation of aniline was increased by 71 and 31%, respectively in lactating rats. Furthermore, the activities of UDP-glucuronyltransferase and glutathione *S*-transferase were increased by 21 and 27%, but those of epoxide hydrolase were reduced by 85%. Western immunoblot analysis of microsomal proteins obtained from pregnant and lactating rats shows that only proteins encoded by the genes of CYP2C6 and CYP3A1 are expressed at detectable levels, whereas the expression of CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2E1 and CYP4A1 was not detectable in pregnant and lactating rats at a protein loading of 3 μg total protein per well. In contrast, in northern blot hybridization experiments, detectable amounts of mRNA of the above named isoenzymes were measurable, but at varying intensities. Based on the northern blot hybridization analysis, an approximate 4-fold and 3-fold increase in CYP2A1 mRNA and CYP3A1 mRNA was found, when lactating rats were compared with female controls or pregnant rats, at day 10 of gestation.

Cytochrome P450 dependent monooxygenases have evolved as a superfamily of haem-containing proteins which catalyse the metabolism of a wide range of endogenous and foreign, i.e. medicinal and industrial chemicals. Changes in the physiological state of an animal may alter its ability to metabolize foreign chemicals.

During pregnancy, gross hormonal changes are noted and it is established that gestation causes a depression in rat hepatic drug metabolism. For example, Turcan *et al.* [1] have shown statistically significant reductions in the metabolism of the substrates *p*-nitrobenzoic acid, aniline and ethylmorphine. These authors have also noted that pregnancy was linked to reductions in the total phospholipid concentrations of microsomal membranes and especially that of phosphatidylcholine. Further studies by Symons *et al.* [2] provided corroborative evidence for a reduced hepatic drug metabolism during pregnancy and it was suggested that these reductions in enzyme activities were linked to a decreased ratio of microsomal phosphatidylcholine to phosphatidylethanolamine and a reduction in the high spin form of ferricytochrome P450.

The early studies by Guarino *et al.* [3] show a lower V_{max} in pregnant rats, when assessed for the P450 catalysed reactions aniline hydroxylation and ethylmorphine N-demethylation. Alterations in the kinetic constants of these reactions were paralleled by a significant lowering in the cytochrome P450 concentration. In addition, Tabei and Heinrichs [4] detail significant reductions in the 7α- and 16α- but not 7β-hydroxylation of dehydroepiandrosterone and a similar reduction in the N-demethylation of aminopyrine was noted for near term animals, i.e. at day 22 of gestation. The influence of phenobarbitone and methylcholanthrene (3-MC§) treatment in pregnant rats was studied by Guenthner and Mannering [5]. These authors found that the phenobarbitone treatment resulted in a 2-fold induction in ethylmorphine N-demethylation when compared with the 4-fold induction observed with non-pregnant females. However, the induction of benzo[*a*]pyrene hydroxylation by 3-MC-treatment was not impaired during pregnancy. Dean and Stock [6] report gestational and post-natal hepatic monooxygenase activities. The greatest reduction in P450 levels, aniline and aminopyrine metabolism was observed at day 20 of gestation. The relative increases in enzyme activities in response to treatment with phenobarbitone (PB) were similar in pregnant and non-pregnant animals. Kardish and

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§ Abbreviations: 3-MC, methylcholanthrene; PB, phenobarbitone; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; UDPGT, UDP-glucuronyltransferase; GST, glutathione *S*-transferase; EROD, ethoxyresorufin *O*-deethylase.

Feuer [7] have shown that the 3-hydroxylation of coumarin and the 16α-hydroxylation of progesterone were significantly reduced during pregnancy. These authors also showed that additions of the progesterones 5β-pregnane-3α, 20α-diol, 5β-pregnane-3α-ol-dione and 5β-pregnane-3,20-dione to microsomal suspensions resulted in a significant reduction in progesterone 16-hydroxylase and coumarin 3-hydroxylase activities.

During pregnancy, high concentrations of progesterone and of its metabolites are present in the blood and the investigations by Kardish and Feuer [7, 8] show that *in vitro* additions of progesterone and its metabolites directly suppress P450 catalysed reactions. The mechanism, however, by which progesterone suppresses microsomal metabolism remains uncertain.

From the above cited studies, it becomes apparent that pregnancy is linked to a reduction in certain P450 catalysed reactions, but the evidence presented is primarily based on measurements of monooxygenases activities. Therefore, hepatic microsomes were isolated from pregnant and lactating rats to measure the metabolism of marker substrates of P450 isoenzymes from families 1 and 2. In addition, western immunoblot analysis was carried out with antibodies raised to CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP2E1, CYP3A1 and CYP4A. The expression of P450 mRNA was also measured in northern blot hybridization experiments.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats were supplied by Charles River Ltd (Margate, U.K.) with a body weight range of 175–200 g. Food and water were provided *ad lib.* The beginning of pregnancy was determined by examination of vaginal plugs. Rats were killed at day 10 and day 20 of gestation and 4 and 14 days post partum.

Preparation of hepatic microsomes and assessment of microsomal and cytosolic enzyme activity. Hepatic microsomes were prepared as described previously [9]. Cytochrome P450 and b5 were measured by the methods of Omura and Sato [10]. Protein concentrations were determined by the method of Lowry *et al.* [11] using bovine serum albumin as the standard. Ethoxyresorufin-O-deethylation activity was determined by the method of Burke *et al.* [12] and the N-demethylation of aminopyrine and dimethylnitrosamine were measured essentially as described by Anderson *et al.* [13]. Aldrin epoxidation was measured by the method of Wolff *et al.* [14] with modifications described previously [15]. The activity of NADPH-cytochrome *c* (P450) reductase, para-hydroxylation of aniline, O-demethylation of nitroanisole and of glucuronyl-transferase using *o*-aminophenol as substrate, were assayed as detailed in Ref. 16 with modifications described previously [15]. Glutathione *S*-transferase (GST) activity was measured by the method of Habig *et al.* [17] using 2.4-dinitro-1-chlorobenzene as a substrate. Epoxide hydrolase was assayed using epichlorohydrin as a substrate with an experimental protocol described by Guengerich and Mason [18].

NADPH-dependent enzyme reactions were assayed by the addition of 12 mM $NADP^+$, 12 mM glucose 6-phosphate, and 60 U of glucose-6-phosphate dehydrogenase in a final volume of 3 mL. Cytochrome P450 catalysed reactions were assayed in a buffer-system containing 0.1 M Tris, pH 7.4; 0.15 M $MgCl_2$ and 0.5 M nicotinamide to prevent the destruction of pyridine nucleotide by tissue nucleosidases.

Kinetic measurements were found to be linear with respect to protein concentration and incubation time. A detailed description of the assays employed and the validation of linear enzyme kinetics is given by Borlakoglu [15].

Western immunoblot analysis. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the method of Laemmli [19], using 9% separating gels. Immunoblotting was performed essentially as described by Towbin *et al.* [20] with modifications according to Lewis *et al.* [21]. After separation, proteins were transferred electrophoretically to nitrocellulose, and probed with various antisera to rat liver cytochrome P450s. Antibodies to the purified enzymes were isolated as described by Wolf *et al.* [22, 23]. These antibodies were used previously and validated in immunoblotting studies with mouse, rat and human microsomal samples. In addition, the isoenzyme specificity of the antisera was demonstrated by immunoblot analysis [24]. Cytochrome CYP4A1 was isolated according to the method of Tamburini *et al.* [25] and was of high purity, running as a single band on SDS–PAGE. Antibodies used were to CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP2E1 (kindly provided by Dr C. W. Yang), CYP3A1 and CYP4A1. A protein loading of 3 μg total protein per well was applied to enable direct comparisons amongst individual groups. Furthermore, the intensity of coomassie blue staining of SDS–PAGE separated proteins was compared to ensure a uniform loading (data not shown).

After visualization of the immunoreactive polypeptides using horseradish peroxidase-labelled second antibody, the signal was enhanced with ^{125}I-protein A (Amersham International plc, Amersham, U.K.) and subsequent autoradiography (Kodak X-Omat AR5 X-ray film) at −70°. Differing exposure times were used in order to optimize the autoradiographic signal.

Northern blot hybridization analysis and mRNA determination. RNA was isolated from liver samples of female rats as described previously by Cox [26]. RNA concentration and purity were estimated spectrophotometrically and tested for integrity and equivalence of loading by ethidium bromide staining of a denaturing formaldehyde gel. mRNA was separated and P450 mRNA content determined on denaturing formaldehyde gels using hybridization conditions described previously by Forrester *et al.* [24]. Blots were washed at 65° with 0.3 M sodium chloride, 0.03 M trisodium citrate, pH 7.4. cDNA probes were labelled by the random-priming method [27–29]. Human cDNA probes used were CYP1A1, CYP2A6, CYP2B1, CYP2E1 (kind gift of Dr F. Gonzalez) and CYP3A3/4. The CYP2B1, CYP2C7 and CYP4A1 (kind gift of Dr G. Gibson [30]) cDNA

Table 1. Liver weight, body weight and microsomal protein concentration of pregnant rats at various timepoints during gestation and after giving birth

Time	A Liver weight (g)	B Body weight (g)	A ×100* B	Microsomal protein (mg/g liver)
Day 10 of gestation (N = 4)	13.20 ± 0.51	298.12 ± 8.94	4.43 ± 0.24	13.89 ± 3.36
Day 20 of gestation (N = 4)	17.07 ± 1.91 P < 0.01	305.31 ± 30.14	5.59 ± 0.25 P < 0.02	16.39 ± 5.70
4 days post partum (N = 4)	14.61 ± 1.87	333.25 ± 14.10	4.38 ± 0.37	43.50 ± 2.12 P < 0.01

Values are means ± SD. * Represents the % contribution of the liver weight to the total body weight.

probes were isolated from rat. Northern blots were rehybridized to actin to adjust for uneven transfer during the blotting procedure. The expression of this gene serves as an internal control for mRNA quantitation during the densitometric analysis of the autoradiographs. Further experimental details are given in Ref. 24.

Chemicals, reagents, enzymic substrates. Unless otherwise stated, all chemicals and reagents were of highest purity and purchased from the Sigma Chemical Co., and from BDH Chemicals, Poole, U.K.

Statistical analysis. Unless otherwise stated values are means ± SD; P-values were determined by Student's *t*-test.

RESULTS

The liver weights, body weights and microsomal protein concentrations are given in Table 1. A significant ($P < 0.01$) 30% increase in liver weight was noted at day 20 of gestation and a significant ($P < 0.02$) 26% increase in the liver to body weight ratio was calculated. Four days post partum, a 17% decline in liver weight was recorded and it is suggested that changes in liver weight are attributable to a transient (hyperthrophic) swelling of parenchymal cells. For comparison, the microsomal protein concentration was more than 3.1- and 2.6-fold increased, when compared with measurements obtained at day 10 and day 20 of gestation (Table 1).

Components of P450 monooxygenases

Pregnancy itself had profound effects on the concentration of hepatic cytochrome P450, cytochrome *b5* and of cytochrome *c* (P450) reductase. As shown in Table 2, the concentrations of cytochrome P450 and cytochrome *b5* were reduced by 33 and 45%, respectively, between day 10 and day 20 of gestation. The activities of NADPH-cytochrome *c* (P450) reductase were marginally reduced between day 10 and day 20 of gestation. The concentrations of cytochrome P450 and cytochrome *b5* and the activities of NADPH-cytochrome *c* (P450) reductase were further reduced by 50, 50 and 23%, respectively, when measurements were compared between day 10 of gestation and 4 days post partum.

The metabolism of marker substrates of CYP450 families 1 and 2 during pregnancy and lactation

The metabolism of the marker substrate aniline, nitroanisole and ethoxyresorufin was measured to investigate CYP1A catalysed reactions (Table 2). The metabolism of these substrates was reduced by 14, 15 and 33%, respectively, at day 20 of gestation. Four days post partum, the rates of nitroanisole O-demethylation and the 4-hydroxylation of aniline were increased by 71 and 31%, respectively, when compared with pregnant rats at day 10 of gestation. During this time point ethoxyresorufin *O*-deethylase (EROD) activities did not differ.

Also, Table 2 details the rates of N-demethylation of aminopyrine and the epoxidation of aldrin during pregnancy and post partum. These measurements allow, at least in part, CYP2B1 catalysed reactions to be assessed. Between day 10 and day 20 of gestation, the rates of N-demethylation of aminopyrine were reduced by 77%, however, the rate of aldrin epoxidation did not differ. In contrast, the rates of aldrin epoxidation and the N-demethylation of aminopyrine were reduced by 74 and 53%, when compared with 10-day-old pregnant rats. The CYP2E1 catalysed N-demethylation of dimethylnitrosamine did not differ between day 10 and day 20 of gestation, but a 20% reduction was measured in 4 day old lactating rats (see Table 2).

Post oxidative drug metabolism during pregnancy and lactation

The activities of UDP-glucuronyltransferase (UDPGT), GST and epoxide hydrolase were assayed to obtain further information on post oxidative drug metabolism. The activities of epoxide hydrolase were reduced between day 10 and day 20 of gestation, whereas those of UDPGT and GST were increased by 17 and 190%, respectively. Furthermore, the activities of UDPGT and GST were increased by 27 and 21%, whereas those of epoxide hydrolase were reduced by 85% in 4-day-old lactating rats when compared with pregnant rats, at day 10 of gestation (Table 3).

Table 2. The effects of pregnancy and lactation on hepatic microsomal monooxygenases

Study number	Animal group	Cytochrome P450 (nmol/mg)	Cytochrome *b5* (nmol/mg)	Cytochrome *c* (P450) reductase (nmol/mg/min)	EROD (pmol/mg/min)	4-Nitroanisole-*O*-demethylase (pmol/mg/min)	4-Hydroxylation of aniline (pmol/mg/min)	Aminopyrine *N*-demethyl. (nmol/mg/min)	Dimethyl-nitrosamine *N*-demethyl. (nmol/mg/min)	Aldrin epoxidation (nmol/mg/min)
1	Day 10 of gestation	0.70 ± 0.16	0.49 ± 0.04	59.7 ± 14.3	52 ± 7	303 ± 70	206 ± 40	5.3 ± 0.5	1.4 ± 0.3	8.4 ± 2.5
2	Day 20 of gestation	0.47 ± 0.23	0.27 ± 0.08*	67.9 ± 15.7	35 ± 5*	259 ± 60	178 ± 28	1.2 ± 0.2*	1.4 ± 0.2	8.7 ± 3.1
3	4 Days post partum	0.35 ± 0.01†	0.25 ± 0.01†	45.8 ± 4.0	56 ± 10§	521 ± 20†§	271 ± 10§	2.5 ± 0.1†§	1.1 ± 0.4	2.2 ± 0.6†§
4	14 Days post partum	0.22 ± 0.13‡	0.20 ± 0.02‡	51.3 ± 2.6	50 ± 10¶	ND	286 ± 20‡‖	2.6 ± 0.3‡‖	ND	ND

Values are means ± SD for N = 4 animals per group; * = 1 vs 2, $P < 0.01$, † = 1 vs 3, $P < 0.01$, ‡ = 1 vs 4, $P < 0.01$, § = 2 vs 3, $P < 0.01$, ‖ = 2 vs 3, $P < 0.01$, ¶ = 2 vs 4, $P < 0.05$.
ND, not determined.

Western immunoblot and northern blot hybridization analysis of CYP450 proteins and mRNA

The results of western immunoblot analysis are shown in Fig. 1. The expression of CYP2C6 and CYP3A1 did not differ in pregnant and lactating rats. Furthermore, CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP4A1 were not detectable in microsomal proteins obtained from pregnant and lactating rats in a protein loading of 3 μg total protein/well.

The nothern blot hybridization analysis is shown in Fig. 2. The mean densitometric measurements associated with isoenzyme-specific P450 mRNA are given in Table 4. It should be noted that the CYP1A cDNA probe hybridized in two mRNA transcripts. The upper band (2.9 kb) was assigned to CYP1A2 mRNA whereas the lower band had a molecular size of 2.2 kb and was assigned to CYP1A1 mRNA. The molecular sizes of these two bands are fully consistent with the values reported by others [31]. An approximate 2-fold increase in CYP1A2 mRNA was found when female control and pregnant rats were compared, but CYP2B1 mRNA was reduced to 50%. Comparisons of densitometric measurements did not indicate significant changes in cDNA hybridized CYP2C7, CYP2E and CYP3A1 mRNA when control females and pregnant rats were compared (Table 4). However, an approximate 4- and 3-fold increase in CYP2A1 and CYP3A1 mRNA was measured when lactating and pregnant rats were compared. Furthermore, the northern blot hybridization analysis provides evidence for the expression of most P450 isoenzymes at the mRNA level, albeit, at varying concentrations. Therefore, the western immunoblot analysis shown in Fig. 1 may have not been sufficiently sensitive to detect the expression of some of the P450 isoenzymes.

DISCUSSION

Previously, several workers have reported changes in hepatic drug metabolism during pregnancy. We are in agreement with the published findings by these authors, but in the present study additional information on the expression of P450 isoenzymes at the protein and mRNA level was obtained. There is evidence for a suppression of hepatic drug metabolism during pregnancy and lactation which is not linked to an altered protein and mRNA expression of P450 isoenzymes.

Reports of hepatic cytochrome P450 measurements of pregnant rats are contradictory, with authors reporting either a decrease in hepatic cytochrome P450 concentrations (Guarino *et al.* [3], Neale and Parke [32], Dean and Stock [33], Feur and Kardish [34], Tabei and Heinrichs [4], Mukhtar *et al.* [35], Dean and Stock [6]) or alternatively an increase in hepatic cytochrome P450 concentrations as denoted by Schlede and Borowski [36], Gut *et al.* [37] and Symons *et al.* [2]. These measurements are based on spectral determination of cytochrome P450.

In the present study, pregnancy was linked to a reduction in oxidative metabolism, but at varying degrees (Table 2). Furthermore, when pregnant and lactating rats were compared, very significant reductions in the concentrations of cytochrome P450

Table 3. The effects of pregnancy and lactation on post oxidative hepatic drug metabolism

Study number	Animal group	Epoxide hydrolase (nmol/mg/min)	GST (nmol/mg/min)	UDPGT (nmol/mg/min)
1	Day 10 of gestation	0.84 ± 0.18	110 ± 10	1.8 ± 0.2
2	Day 20 of gestation	0.68 ± 0.28	210 ± 31*	2.1 ± 0.1**
3	4 Days post partum	0.13 ± 0.01†§	134 ± 24§	2.3 ± 0.1
4	14 Days post partum	0.24 ± 0.13‡¶	173 ± 39‖	2.8 ± 0.3†††

Values are means ± SD for N = 4 animals per group, * = 1 vs 2, $P < 0.01$, † = 1 vs 3, $P < 0.01$, ‡ = 1 vs 4, $P < 0.01$, § = 2 vs 3, $P < 0.01$, ‖ = 1 vs 4, $P < 0.05$, ¶ = 2 vs 4, $P < 0.05$, ** = 1 vs 2, $P < 0.05$, †† = 3 vs 4, $P < 0.05$.

and cytochrome *b5* were measurable. As a consequence, the activities of certain isoenzymes of the CYP2 family were up to 74% (see Table 2) reduced. In contrast, the activities of isoenzymes of the CYP1 family did either not differ (EROD) or were increased (nitroanisole O-demethylation, 4-hydroxylation of aniline). Moreover, unlike epoxide hydrolase, the activities of UDPGT and GST were increased during pregnancy and in lactating rats, and similar results were obtained when pregnant rats at day 10 of gestation were compared with rats, 4 days post partum.

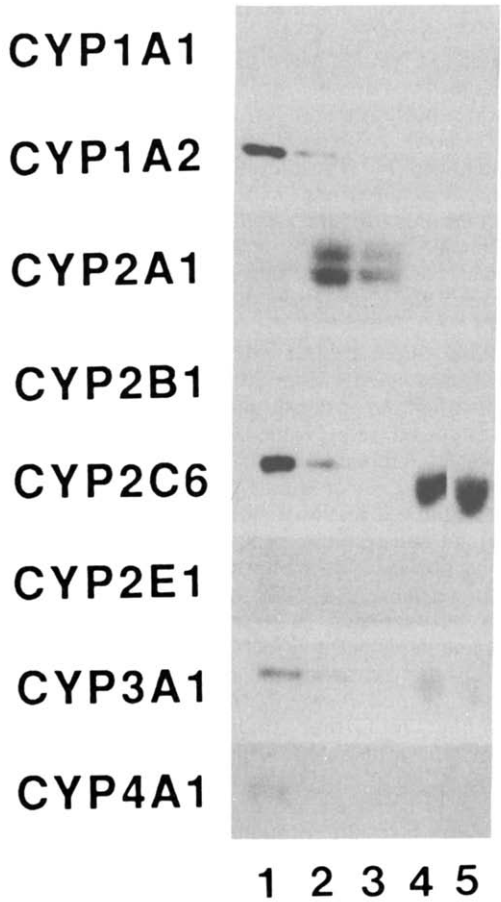

Fig. 1. Western blot analysis of microsomal P450 proteins isolated from livers of pregnant and lactating mothers. Microsomal proteins were separated by SDS–PAGE, transferred to nitrocellulose and probed with anti-P450 antisera, as detailed in Materials and Methods. Lanes 1 to 3 standards (1.2, 0.8 and 0.4 pmol purified P450, respectively, with the exception of CYP2A1, only 0.8 and 0.4 pmol were used). Control pregnant mothers lane 4. Control lactating mothers lane 5.

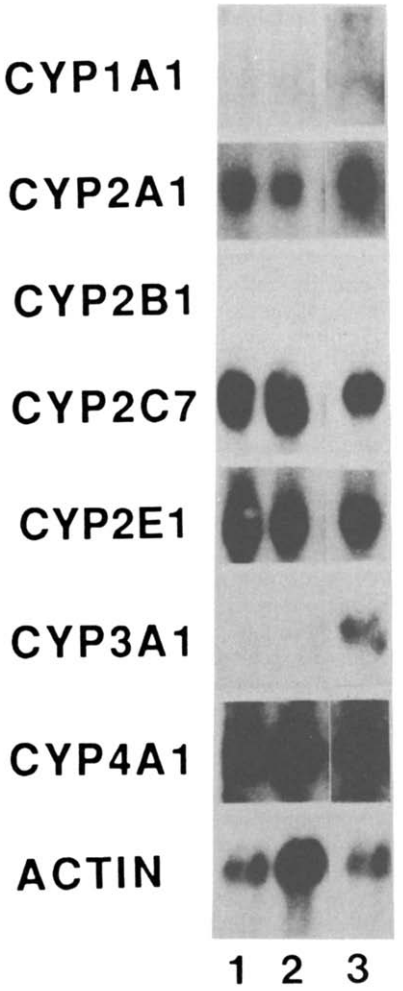

Fig. 2. Cytochrome P450 mRNA levels in liver samples isolated from pregnant and lactating mothers. RNA (10 μg) of maternal and foetal liver samples was separated on denaturing formaldehyde agarose gels, transferred to Hybond N and probed with P450 cDNA probes as outlined in Materials and Methods. Lane 1 control females. Lane 2 pregnant mothers. Lane 3 lactating mothers.

Table 4. Mean densitometric signals of areas associated with isoenzyme specific mRNA

	Control female	Pregnant	Lactation	Molecular size of cDNA probe (kb)
CYP1A1	160	132	ND	2.2
CYP1A2	133	272	ND	2.9
CYP2A1	2301	1927	8097	2.0
CYP2B1	385	191	ND	1.8
CYP2C7	18343	20966	24217	2.0
CYP2E1	5260	6320	4781	1.8
CYP3A1	794	1912	6332	1.8
CYP4A1	6216	10166	8367	2.1

ND, not determined, values are means of N = 3 independent scannings.

A depression in hepatic Phase I and II drug metabolism has been attributed to changes in the microsomal phospholipid environment [2] with a significantly decreased ratio of phosphatidylcholine to phosphatidylethanolamine during gestation. This appears to be particularly important since Strobel *et al.* [38] have shown that phosphatidylethanolamine inhibited the phosphatidylcholine stimulated hydroxylation of benzphetamine. Moreover, Turcan *et al.* [1] and Symons *et al.* [2] have shown a significant decrease in the high spin form of ferricytochrome P450, and it has been suggested that changes in the spin-state equilibrium of cytochrome P450 may modulate monooxygenase activities.

There is an impressive body of evidence to suggest that hormonal changes during pregnancy, and in particular high plasma concentrations of progesterone and of its metabolites, are directly linked to a depressed hepatic drug metabolism. Previous reports [7, 8] have shown evidence of reduced hepatic drug metabolism during pregnancy, with significant reductions in the 3-hydroxylation of coumarin and the 16α-hydroxylation of progesterone. Interestingly, in *in vitro* experiments, CYP2B1 and CYP2B2 exclusively metabolized progesterone to its 16α-hydroxylated product. *In vitro* addition (1.6–80 μM) of dilauroylphosphatidylcholine inhibits the formation of this metabolite [39]. Nevertheless, progesterone is extensively and regioselectively metabolized by 10 different isoenzymes [40]. The studies by Kardish and Feuer [7] have shown that *in vitro* additions of the progestagens 5β-pregnane-3α-20α-diol, 5β-pregnane-3α-ol-zoone and 5β-pregnane-3,20-dione caused a significant reduction in progesterone 16α-hydroxylase and coumarin 3-hydroxylase activities. These results imply that the above listed progestagens are likely to suppress the catalytic activities of certain monooxygenases, when assessed for progesterone metabolism. However, further experimentation is required to elucidate the precise mechanism of the inhibitory effect of these progestagens, and to understand to what extent hormonal changes during pregnancy are directly linked to a suppressed hepatic drug metabolism. This appears to be particularly important since Dean and Stock [33] have shown that hepatic microsomal concentrations of progesterone were not significantly increased during pregnancy.

In the present study, the expression of CYP2C6 and CYP3A1 did not differ in pregnant and lactating rats. Furthermore, CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP4A1 were not detectable in microsomal proteins obtained from pregnant and lactating rats in a protein loading of 3 μg total protein/well. This is, however, in good agreement with the reports summarized by Gonzalez [31] as the latter P450 isoenzymes are not expressed at high tissue concentrations in untreated (control) animals, but are highly inducible by a wide range of chemical agents. Nevertheless, the northern blot analysis shown in Fig. 2 evidences the presence of CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C7, CYP2E1, CYP3A1 and CYP4A1 mRNA transcripts, albeit, at varying concentrations. Marginal differences were found when mean densitometric counts of controls and pregnant rats (Table 2) were compared, but significant increases in CYP2A1 and CYP3A1 mRNA were detected post partum. This suggests a differential expression of certain P450 mRNAs in pregnant and lactating rats, however, contrasts the results obtained by western immunoblot analysis. As shown in Fig. 1, CYP2A1, CYP2E1 and CYP4A1 were not detected in microsomal proteins from pregnant and lactating rats, but in northern blot hybridization experiments large amounts of their respective mRNA was found (see Table 4). It is evident that further study will be needed to understand in detail these differences.

In conclusion, our studies do not provide evidence for a coordinate change in the expression of P450 isoenzymes at the protein and mRNA level to account for the changes in hepatic drug metabolism during pregnancy and lactation.

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