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DRUG METABOLISM, CYTOCHROME P450 SPIN STATE, AND PHOSPHOLIPID CHANGES DURING PREGNANCY IN THE RAT

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Pregnancy in the rat is accompanied by a decrease in hepatic microsomal drug metabolism [1, 2]. In this paper we report the relationships between microsomal drug metabolism, phospholipids and the ferric spin state of cytochrome P-450.

METHODS

Mated female Wistar albino rats (180 ± 10g) were kept under controlled conditions on Steralit bedding. Twenty day pregnant rats were used as experimental animals, non-pregnant rats being used as controls.

Preparation of liver fractions. The 10,000*g* supernatant was prepared by the method of Neale and Parke [1]. Microsomes were prepared by the method of Elcombe *et al.* [3].

Enzyme assays. Ethylmorphine *N*-demethylase [4], aniline *p*-hydroxylase [5] and *p*-nitrobenzoic acid reductase [6] were determined in the 10,000*g* supernatant fraction by standard procedures.

Protein and phospholipid analysis. Microsomal protein was determined by the method of Miller [7] and cytochrome P450 according to Sladek and Mannering [8]. Microsomal phospholipids were extracted according to the method of Folch *et al.* [9] and individual components separated by the method of Cooper and Feuer [10] using the solvent system, chloroform (50): methanol (25): glacial acetic acid (7): water (3). Spots were visualised with iodine vapour. Phospholipids were quantified by a molybdate/vanadate colour reaction (Boehringer Mannheim GmbH Test Kit Cat No. 124974).

Hemoprotein spin state analysis. Thermally induced spin state transitions were recorded on a Perkin–Elmer 356 spectrophotometer operating in the dual-wavelength mode. Microsomes were diluted (to a concentration of 1 nmole cytochrome P450/ml) in 50 mM phosphate buffer pH 7.25, containing 20% (v/v) glycerol and 10 mM EDTA. The suspension was pipetted into a cuvette and placed in the spectrophotometer with the monochromators fixed at 390 and 420nm. A thermistor temperature probe (Type FM, Edale Instruments, Cambridge, England) was placed in the cuvette and connected to a temperature readout device with a full scale expansion of 0 to 50° (Model C, Edale Instruments, Cambridge, England). The temperature of the cuvette was raised from 14 to 40° and the increase in ΔA (390 minus 420nm) recorded at 2° increments. The absorbance values of cytochrome P450 corresponding to 100% low spin material and 100% high spin material were used as free-floating parameters in conjunction with the observed ΔA values in a one parameter fit procedure described by the equation $k = A_{LS}\text{-}A/A\text{-}A_{HS}$, assuming $A_{HS} - A_{LS} = 126 mM^{-1}cm^{-1}$ [11, 12]

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Table 1. Hepatic microsomal enzyme activities and phospholipids in pregnant (20 days) and non-pregnant rats

	Non-pregnant	Pregnant
Ethylmorphine *N*-demethylase (μmoles/g liver/hr)	9.9 ± 0.15	7.3 ± 0.20‡
Aniline hydroxylase (μmoles/g liver/hr)	1.20 ± 0.02	0.88 ± 0.01‡
p-Nitrobenzoic acid reductase (μmoles/g liver/hr)	1.84 ± 0.03	0.98 ± 0.08‡
Cytochrome P450 (nmoles/mg protein)	0.70 ± 0.02	0.71 ± 0.21
Total phospholipids (μmoles/g liver)	13.9 ± 0.4	11.4 ± 0.2 †
Phosphatidylcholine (μmoles/g liver)	8.33 ± 0.16	6.47 ± 0.10‡
Phosphatidylethanolamine (μmoles/g liver)	2.70 ± 0.09	3.64 ± 0.08‡

Results are expressed as mean ± S.E.M. ($n = 4$). Significant differences (Student's *t*-test) are shown as *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

where k is the spin equilibrium constant (defined as the ratio of high spin to low spin cytochrome P450), A_{LS} the delta absorbance of 100% low spin cytochrome P450, A_{HS} the delta absorbance of 100% high spin material and A is the observed ΔA (390 minus 420 nm) at any given temperature. The spin equilibrium constant was fitted to a Van't Hoff plot by reiterative analysis [11] on line to the University of Surrey Computing Centre.

RESULTS

There was a significant decrease in the activity of the hepatic mixed function oxidase system in 20 day pregnant rats compared to non-pregnant controls (Table 1). This decrease was greatest for *p*-nitrobenzoic acid reductase (53% of non-pregnant levels) and least for ethylmorphine *N*-demethylase (74% of non-pregnant levels). Microsomal concentrations of total cytochrome P450 were unchanged. Both total phospholipid and phosphatidylcholine were significantly decreased (82% and 78% of non-pregnant levels respectively). The phosphatidylcholine: phosphatidylethanolamine ratio was altered from 3:1 in non-pregnant rats to less than 2:1 in the pregnant rats. The microsomal content of high spin cytochrome P450 was calculated from Van't Hoff plots relating the spin equilibrium constant (k) to temperature and is shown in Table 2.

During pregnancy there was a significant decrease in the proportion of cytochrome P450 in the high spin state at both 20° and 37°.

DISCUSSION

The results of this study confirm earlier reports that the activity of the hepatic microsomal mixed function oxidase system is decreased in late pregnancy [1, 2]. Microsomal concentrations of cytochrome P450 however, remain unchanged (Table 1) and cannot explain the changes observed in drug metabolism. This finding is in agreement with previous work [13, 14], although others have reported a decrease in cytochrome P450 [2, 15]. Present results suggest that changes in phospholipids and/or the hemoprotein spin state may be responsible for the decrease in drug metabolism.

The importance of phospholipids in mixed function oxidase activity is well established [16, 17]. Phospholipids are important in substrate binding [18], electron transfer to cytochrome P450 [19], and maintaining the hemoprotein and NADPH-cytochrome P450 reductase in a functional complex. Guengerich and Coon [20] have shown that phosphatidylcholine lowered K_s and K_m for benzphetamine, and increased both ΔA_{max} and V_{max}. Tsong and Yang [21] have suggested that phospholipids regulate substrate-induced conformational change of cytochrome P450. The decrease in total microsomal phospholipid and the altered phosphatidylcholine: phosphatidylethanolamine ration seen in pregnancy might therefore explain the decrease in cytochrome P450-mediated drug metabolism. The increase in phosphatidylethanolamine in pregnancy (Table 1) may be important as phosphatidylethanolamine inhibits the phosphatidylcholine-enhanced hydroxylation of benzphetamine [17].

The decrease in high spin cytochrome P450 in pregnancy may also explain the decrease in drug metabolism. The rate limiting step for the oxygenation of a number of drugs is the first electron reduction of cytochrome P450 by NADPH–cytochrome P450 reductase [22]. The spin state of the hemoprotein is important with respect to the ease of this reduction since an increase in the high spin form of cytochrome P450 is associated with a positive shift in the redox potential thus facilitating electron flow from the reductase to the terminal cytochrome [23].

The change in hemoprotein spin state induced by pregnancy may be associated with high concentrations of steroids, particularly progesterone, circulating at this time. Further work in this laboratory however, has revealed that the decrease in drug metabolism is not related to increased steroid levels. Furthermore, hepatic microsomal concentrations of progesterone were not significantly increased in the rat during pregnancy [2]. Spin state changes in pregnancy might alternatively be related to microsomal phospholipid changes. To date, there have been no reports on the effect of changes in microsomal phospholipid levels on the spin state of cytochrome P450. Gibson *et al.* [12] found that the addition of a microsomal lipid extract to purified cytochrome P450 caused an increase in the high spin form of the hemoprotein from 17% to 53% at 20°. This change was attributed to microsomal free fatty acids.

We suggest that the decrease in drug metabolism during pregnancy is related to an altered membrane phospholipid environment and/or a decrease in the high spin character of cytochrome P450, which might be modulated by the change in microsomal phospholipids.

Table 2. Spin state of hepatic microsomal cytochrome P450 from pregnant (20 days) and non-pregnant rats

Temp °C	Percentage high spin cytochrome P450	
	Non-pregnant	Pregnant
20	54.5 ± 1.1	43.2 ± 1.6†
37	73.6 ± 1.7	61.3 ± 2.0†

The percentage high spin is based on the equilibrium constant $k = (P450_{HS})/(P450_{LS})$. Mean values ± S.E.M. for 4 animals are shown. These values are the percentages of that part of the total P450 which undergoes a temperature-dependent change in spin state. Significant differences (Student's t test) are shown as *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

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