Biochemical Pharmacology, Vol. 26, pp. 1495–1499. Pergamon Press, 1977. Printed in Great Britain.

DIFFERENTIAL ACTION OF PROGESTERONES ON HEPATIC MICROSOMAL ACTIVITIES IN THE RAT

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(*Received* 11 *September* 1976; *accepted* 17 *December* 1976)

Abstract—A significant reduction was found in the activity of drug-metabolizing enzymes (aminopyrine *N*-demethylase and coumarin 3-hydroxylase) and glucose 6-phosphatase in hepatic microsomes after the administration of reduced derivatives of progesterone (5α-pregnane-3β-ol-20-one, 5β-pregnane-3α-ol-20-one, 5α-pregnane-3β,20β-diol and 5β-pregnane-3α,20α-diol) to rats. These steroids slightly raised inosine diphosphatase activity. On the other hand, 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile significantly increased drug metabolism and slightly elevated glucose 6-phosphatase. The contrasting action of the different progesterone derivatives was associated with changes in microsomal phospholipid synthesis. Pregnanolone and pregnanediol significantly decreased the *de novo* incorporation of [^{14}C-Me]-L-methionine into microsomal phospholipids, mainly manifesting in phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine fractions; reduced the activity of *S*-adenosyl-L-methionine:microsomal-phosphatidylethanolamine methyl transferase; and caused a reduction of total microsomal phosphatidylcholine:phosphatidylethanolamine ratio. In contrast, 16α-hydroxy-progesterone and pregnenolone-16α-carbonitrile increased the *de novo* synthesis of microsomal phospholipids, methyl transferase activity and the ratio of total microsomal phosphatidylcholine: phosphatidylethanolamine. Treatment of rats with reduced progesterone derivatives diminished microsomal progesterone hydroxylation in the 16α- and 6β-position and raised progesterone Δ^4-5α-dehydrogenase activity measured *in vitro*. On the other hand, 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile elevated progesterone hydroxylation. Considering these opposite effects it can be postulated that in the rat the induction of drug-metabolizing activity of the hepatic endoplasmic reticulum might be controlled by a balance displayed in the synthesis and metabolism of various progesterone derivatives.

Reports on the regulation of drug metabolism by various steroids in the rat liver have revealed opposite findings. Progestational steroids (progesterone and norethynodrel [1] and medroxyprogesterone acetate [2]) and combination contraceptives (medroxyprogesterone-ethynyl estradiol or mestranol [2], norethynodrel-mestranol [3], and quingestanol acetate-ethynyl estradiol [4]) increased *N*-demethylation and other microsomal drug-metabolizing activity. Catatoxic steroids (spironolactone [5, 6] and pregnenolone-16α-carbonitrile [7, 8]), estrogens (ethyl-estrenol and norbolethone [5]) and anabolic steroids (testosterone propionate, 19-nortestosterone [9] and methyltestosterone [6, 10] also stimulated drug metabolism in the female rat. In contrast, several steroids were found to reduce drug metabolism; norethynodrel and norethindrone [11] increased pentobarbital sleeping time and decreased the metabolism of several drugs *in vivo*. Estradiol, diethylstilbestrol, testosterone and androsterone brought about competitive inhibition [12] *in vitro*. Similar effects were found with norethynodrel [1] and norethindrone on drug [11] and estrogen [13] metabolism. The contradictory results are partly due to different dose levels applied; very high doses of steroids inhibited drug-metabolizing activity in the liver [1, 11], lower but still high levels elicited no change [2, 11], or slight elevation [14], whereas low doses increased drug metabolism *in vivo* [1, 15]. Human data are also conflicting [3, 16, 17] using oral contraceptives.

Recent studies have shown that hepatic microsomal hydroxylation is less effective during pregnancy [18–20]. The reduced drug metabolism in the pregnant animal and the delayed development in the newborn [21, 22] might be associated with the presence of elevated amounts of reduced progesterone metabolites. Since drugs and steroids are hydroxylated by common enzyme systems bound to liver microsomes [23, 24], it has been postulated that changes in normal steroid balance could influence the metabolism of drugs and the pattern of liver response.

In order to test this hypothesis, we have administered various reduced and oxidized progesterone derivatives: 5α- and 5β-pregnane-3α,20α-diol, 5α- and 5β-pregnane-3α-ol-20-one, 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile to rats. The effect of these compounds on the microsomal hydroxylation of selected drugs and of progesterone has been measured.

MATERIALS AND METHODS

Wistar female rats weighing 200–250 g (High Oak Ranch, Richmond Hill, Ontario) were used in these experiments. Animals received 5α-pregnane-3β-ol-20-one, 5β-pregnane-3α-ol-20-one, 5α-pregnane-3β,20β-diol, 5β-pregnane-3α,20α-diol (Sigma Chemical Co., St. Louis, MO), progesterone-16α-carbonitrile (Upjohn Co., Kalamazoo, MI) or 16α-hydroxy-progesterone. The latter compound was synthesized according to

a published procedure [25]. Test compounds were injected s.c. in seven daily 20 mg/kg doses dissolved in arachis oil. Control groups received the vehicle only. The last dose was given 24 hr before sacrifice. [^{14}C-Me]L-Methionine, 25 μCi (sp. act. 33 mCi m-mole, International Chemical and Nuclear Corp., Montreal), was dissolved in 0.2 ml physiological saline solution and injected i.p. 1 hr before sacrifice.

Rats were killed under light anaesthesia by exsanguination via the inferior vena cava. Various methods have been used as established in the literature or in our laboratory for the preparation of liver homogenates and microsomal fractions [26, 27]; determination of aminopyrine *N*-demethylase [28], coumarin 3-hydroxylase [29], glucose 6-phosphatase [27] and *S*-adenosyl-L-methionine:microsomal-phosphatidylethanolamine methyl transferase (methyl transferase) [30] activities; metabolism of progesterone [22] using [4-^{14}C]progesterone (sp. act. 52.8 mCi/m-mole, New England Nuclear, Boston, MA); incorporation of [^{14}C-Me] groups from [^{14}C-Me]L-methionine [31] extraction, purification and quantitation of microsomal phospholipids [31–33]; and determination of phosphate [34] and protein contents [35]. Radioactivity of phospholipid fractions was measured using a Packard Tri-Carb liquid scintillation counter.

Results were analysed statistically by the Student's *t*-test [36] and significance was accepted at $P < 0.05$ level.

RESULTS

Effect on microsomal drug metabolism. Treatment of rats with 5α- or 5β-pregnane-3α-ol-20-one and 5α- or 5β-pregnane-3α,20α-diol caused a reduction of aminopyrine *N*-demethylase and coumarin 3-hydroxylase activities. In contrast, pregnenolone-16α-carbonitrile and 16α-hydroxyprogesterone brought a significant increase of these enzyme activities (Table 1).

Effect on microsomal phosphatase. Reduced progesterone metabolites (5α- or 5β-pregnane-3α-ol-20-one, and 5α- or 5β-pregnane-3α,20α-diol) decreased glucose 6-phosphatase. 16a-Hydroxyprogesterone or pregnenolone-16α-carbonitrile treatments caused a marked increase (Table 2).

Effect on microsomal phospholipid synthesis. Various progesterone derivatives elicited an opposite action on the *de novo* production of microsomal phospholipid. Incorporation of [^{14}C-Me]methionine was significantly inhibited by 5α- or 5β-pregnane-3α-ol-20-one and 5α- or 5β-pregnane-3α,20α-diol treatments, whereas 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile elicited an increase (Table 3). These changes were manifest in phosphatidylcholine (PC), phosphatidylethanolamine (PE) and lysophosphatidylcholine (LPC) fractions. The radioactivity in the latter represented phosphatidylmono- and diethanolamines [37]. Other phospholipid fractions remained unaltered by these treatments. Methyl transferase activity was diminished by the reduced progesterones and enhanced by the 16α-hydroxy derivative and the carbonitrile (Table 4).

Significant effects were not found on total microsomal phospholipid content by the various treatments with the exception of pregnenolone-16α-carbonitrile. This test compound and 16α-hydroxyprogesterone significantly increased PC fractions. Reduced progesterone derivatives brought about a moderate decrease of PC content and raised the PE level. Thus, there was a shift in the relative amounts of PE and PC fractions (Table 5), corresponding to changes that followed the uptake of [^{14}C-Me]methionine (Table 3).

Effect on microsomal progesterone metabolism. Reduced progesterone derivatives caused a decrease of progesterone hydroxylation in the 16α- and 6β-position, while 16α-hydroxyprogesterone of pregnenolone-16α-carbonitrile treatment increased the activity of these hydroxylases measured *in vitro*. On the other hand, progesterone Δ^4-5α-dehydrogenase activity was significantly enhanced by the administration of reduced derivatives (Table 6).

DISCUSSION

Studies in recent years have shown that drug-metabolizing enzymes, which are bound to liver microsomes, catalyzed the metabolism of steroids and other endogenous body constituents [23, 24]. The administration of an inducer to experimental animals, therefore, not only stimulated the biotransformation of drugs, but also raised the metabolism of many steroid hormones [38]. For instance, increased activity of progesterone hydroxylase induced by the administration of drugs enhanced progesterone metabolism and consequently reduced its tissue level and pharmacolo-

Table 1. Drug-metabolizing activity in hepatic microsomes of rats given various progesterone derivatives*

Treatment	Coumarin 3-hydroxylase (μmoles/hr/mg protein)	Aminopyrine demethylase (nmoles/hr/mg protein)
Control	12.53 ± 0.91	115.67 ± 6.24
5α-Pregnane-3β-ol-20-one	8.25 ± 0.75†	90.80 ± 5.13†
5α-Pregnane-3β,20β-diol	8.43 ± 0.36†	92.73 ± 2.11†
5β-Pregnane-3α-ol-20-one	7.68 ± 0.42†	
5β-Pregnane-3α,20α-diol	8.01 ± 0.33†	
16α-Hydroxyprogesterone	16.67 ± 0.47†	148.66 ± 7.49†
Pregnenolone -16α-carbonitrile	23.45 ± 1.18†	166.55 ± 8.87†

* Each compound was administered s.c. to female rats in seven daily doses of 10 mg/kg. The results represent the mean ± S. E. of four animals in each group.
† $P < 0.05$ compared to the control group.

Table 2. Phosphatase activity in hepatic microsomes of rats given various progesterone derivatives*

Treatment	Glucose 6-phosphatase (μmoles/hr/mg protein)	Inosine di-phosphatase (μmoles/hr/mg protein)
Control	3.78 ± 0.33	3.58 ± 0.22
5α-Pregnane-3β-ol-20-one	2.95 ± 0.37†	4.28 ± 0.15
5α-Pregnane-3β,20β-diol	3.12 ± 0.17†	4.54 ± 0.11
5β-Pregnane-3α-ol-20-one	2.88 ± 0.22†	
5β-Pregnane-3α,20α-diol	3.05 ± 0.30†	
16α-Hydroxyprogesterone	4.02 ± 0.28	3.75 ± 0.15
Pregnenolone-16α-carbonitrile	4.46 ± 0.54	3.89 ± 0.31

* For treatment details see first footnote in Table 1.
† $P < 0.05$ compared to the control group.

gical action [24, 39]. Conversely, the treatment of rats with various steroids altered the ability of liver microsomes to metabolize drugs [4, 40].

In present investigations between reduced and hydroxy progesterone derivatives, opposite actions have been observed on hepatic drug-metabolizing enzymes, microsomal phosphatases, metabolism of progesterone itself and on the synthesis of microsomal phospholipids essential to the function of these enzymes. The effects of pregnanolone and pregnanediol indicated a trend to inhibit the activity of hydroxylases, increase dehydrogenase and reduce glucose 6-phosphatase, microsomal phospholipid content and production. There appeared to be no difference in action between the allo (5α) and epi (5β) stereoisomers representing rat or human steroid hormones respectively. In contrast to the reduced progesterone derivatives, 16α-hydroxyprogesterone elevated hydroxylases, microsomal phospholipid level and synthesis. Pregnenolone-16α-carbonitrile elicited similar action to those of 16α-hydroxy-progesterone, in agreement with published reports [7, 8]. The oral administration of steroids to women did not alter drug metabolism consistently [3]; their action may be inhibitory [16, 17]. This may be due to the great difference existing between human and animal experiments. In animal experiments, the steroid ranged between 10 and 50 mg/kg/day, whereas in the combination contraceptive therapy 5 mg norethynodrel and 0.075 mg mestranol were taken. Nevertheless, this observation indicated that the effect of steroids on experimental animals cannot be simply extrapolated to man.

Several progesterone derivatives have been tested on drug metabolism in rat as well as in women *in vivo* and *in vitro* [12, 41–44]. The animal studies that pregnanolone, pregnanedione and pregnanediol caused inhibition among most conditions studied. Progesterone itself only elicited slight inhibitory action. The presence of hydroxyl group in the 17α-position in progesterone, pregnenolone or pregnanediol reduced the inhibitory effect or even rendered the steroid inactive. Contradictory findings reported in the literature [1–4, 9–15] might be due to differences in dose levels [1, 2, 11, 14, 15] or a reflection of the metabolism of the test steroid to produce hydroxylated and reduced derivatives.

Previous studies from our laboratory have shown that the changes brought about by reduced progesterones on drug metabolism were similar to the effect of pregnancy [20] which is associated with an increased production of these hormones. The delayed development of drug metabolism of the liver in the newborn is probably a reflection of the circulating maternal progesterones [22]. In the present study, it was demonstrated that the decrease of hydroxylase activities and related parameters characteristic of the action of pregnenolone and pregnanediol was reversed by the administration of hydroxyprogesterone. The opposing response to these steroids raised the possibility that in the rat liver various steroids

Table 3. Incorporation of [^{14}C-Me]L-methionine into phospholipids in hepatic microsomes of rats given various progesterone derivatives*

Treatment	Incorporation of [^{14}C-Me]methionine into microsomal phospholipids (dis./min × 10^3/g liver)			
	Total	PC	PE	LPC
Control	207.52 ± 10.61	187.36 ± 10.77	0.85 ± 0.08	3.78 ± 0.18
5α-Pregnane-3β-ol-20-one	178.60 ± 13.73†	149.50 ± 5.57†	0.80 ± 0.05	2.98 ± 0.22
5α-Pregnane-3β,20β-diol	168.40 ± 8.73†	162.10 ± 7.11†	0.87 ± 0.05	3.25 ± 0.34
5β-Pregnane-3α-ol-20-one	160.94 ± 7.54†	164.16 ± 5.92†	0.85 ± 0.03	3.17 ± 0.12
5β-Pregnane-3α,20α-diol	177.11 ± 5.52†	143.99 ± 4.42†	0.73 ± 0.04	2.98 ± 0.11
16α-Hydroxyprogesterone	252.85 ± 15.20†	229.51 ± 8.52†	0.92 ± 0.03	4.63 ± 0.38
Pregnenolone-16α-carbonitrile	242.81 ± 11.26†	219.61 ± 6.16†	0.90 ± 0.06	4.43 ± 0.45

* For treatment details see first footnote in Table 1.
† $P < 0.05$ from the control group.

Table 4. *S*-Adenosyl-L-methionine; microsomal-phosphatidylethanolamine methyl transferase activity in hepatic microsomes of rats given various progesterone derivatives*

Treatment	*S*-Adenosyl methionine methyl transferase (nmoles/hr/mg protein)
Control	0.0998 ± 0.0016
5α-Pregnane-3β-ol-20-one	0.0834 ± 0.0010
5α-Pregnane-3β,20β-diol	0.0865 ± 0.0010†
5β-Pregnane-3α-ol-20-one	0.0859 ± 0.0009†
5β-Pregnane-3α,20α-diol	0.0879 ± 0.0013†
16α-Hydroxyprogesterone	0.1104 ± 0.0049†
Pregnenolone-16α-carbonitrile	0.1042 ± 0.0054

* For treatment details see first footnote in Table 1.
† $P < 0.05$ from the control group.

could serve as regulators of drug metabolism irrespective of their pharmacological or endocrinological properties. A balance between hydroxy- and reduced progesterones might control some metabolic function of the endoplasmic reticulum related to drug biotransformation. A similar role might be played by the anabolic and catabolic steroids, since some of them caused inhibition and deteriorating action on the endoplasmic reticulum, while others induced drug metabolism [1, 2, 45, 46]. The lower drug-metabolizing activity of the female rat [43] associated with a reduced microsomal phospholipid synthesis [30] as compared to males might also be related to the presence of pregnenolone and pregnanediol.

The endoplasmic reticulum membrane constitutes a protein–phospholipid complex. In this complex the protein moiety is responsible for enzyme activity and phospholipids are essential in their catalytic function [47]. We have suggested that the phospholipid component played an important role in the formation of the endoplasmic reticulum membranes and in the induction or inhibition processes brought about by foreign compounds [30, 31]. The amount of microsomal phospholipids, in particular PE and PC contents, seemed to be associated with the activity of drug-metabolizing enzymes. Modification of phospholipid composition influenced the base level of hepatic drug metabolism; the production of more PC relative to PE brought about an increased enzyme level. Reduction of the PC to PE ratio and the degradation to LPC were associated with a decrease. A casual relationship has been suggested in newborn rodents between the low activity of the mixed function oxidase system and increased phospholipid synthesis and content [48]. Elevated amounts of microsomal phospholipids, in particular their unsaturated side chains, were considered to be the major contributing factors in the inhibitory action [49]. This observation could not exclude our proposal by emphasizing the importance of microsomal PC synthesis from PE by stepwise methylation as an essential process in the regulation of drug metabolism. Since 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile increased enzyme activity and enhanced PC production while pregnenediol and pregnanolone decreased these processes, the action of various steroids on drug metabolism is probably associated with the synthesis of membrane-bound phospholipid.

Table 5. Phospholipid changes in hepatic microsomes of rats given various progesterone derivatives*

Treatment	Microsomal phospholipid (μmoles P/g liver)			
	Total	PC	PE	LPC
Control	6.44 ± 0.19	4.38 ± 0.20	1.08 ± 0.07	0.20 ± 0.02
5α-Pregnane-3β-ol-20-one	6.13 ± 0.17	3.88 ± 0.18	1.15 ± 0.05	0.21 ± 0.01
5α-Pregnane-3β,20β-diol	6.28 ± 0.11	4.08 ± 0.11	1.19 ± 0.07	0.18 ± 0.02
5β-Pregnane-3α-ol-20-one	6.08 ± 0.28	3.52 ± 0.18	1.19 ± 0.11	0.17 ± 0.011
5β-Pregnane-3α,20α-diol	6.02 ± 0.18	3.81 ± 0.11	1.18 ± 0.07	0.18 ± 0.015
16α-Hydroxyprogesterone	6.51 ± 0.08	5.39 ± 0.21†	1.09 ± 0.08	0.21 ± 0.02
Pregnenolone 16α-carbonitrile	8.30 ± 0.30†	5.44 ± 0.30†	1.15 ± 0.05	0.29 ± 0.02

* For treatment details see first footnote in Table 1.
† $P < 0.05$ from the control group.

Table 6. Activity of some progesterone-metabolizing enzymes in hepatic microsomes of rats goven various progesterone derivatives*

Treatment	Progesterone-metabolizing enzymes (nmoles/hr/mg protein)		
	16α-Hydroxylase	6β-Hydroxylase	Δ^{14}-5α-Hydrogenase
Control	10.85 ± 0.84	0.722 ± 0.075	19.08 ± 2.08
5α-Pregnane-3β-ol-20-one	8.12 ± 0.55†	0.578 ± 0.016	27.75 ± 5.19
5α-Pregnane-3β,20β-diol	8.04 ± 0.23†	0.692 ± 0.039	26.19 ± 3.89
5β-Pregnane-3α-ol-20-one	5.52 ± 0.28†	0.588 ± 0.015	21.58 ± 3.98
5β-Pregnane-3α,20α-diol	6.87 ± 0.28†	0.572 ± 0.022	27.37 ± 3.08
16α-Hydroxyprogesterone	16.03 ± 0.22†	0.910 ± 0.084	21.13 ± 2.19
Pregnenolone-16α-carbonitrile	21.59 ± 0.97†	0.952 ± 0.045†	18.15 ± 2.77

* For treatment details see first footnote in Table 1.
† $P < 0.05$ from the control group.

Acknowledgements—This investigation was supported by the Medical Research Council and the Alcoholism and Drug Research Foundation to whom our thanks are due.

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