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FURTHER STUDIES ON THE INHIBITION OF DRUG METABOLISM BY PREGNANOLONE AND RELATED STEROIDS*

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Abstract—Because of our previous findings that pregnanolone and related metabolites of progesterone inhibit drug demethylation and hydroxylation reactions *in vitro*, a variety of other steroids and naturally occurring substances were tested for inhibition of the *O*-demethylation of *p*-nitroanisole by 9000 *g* hepatic supernatants from adult male rats. Of some 58 steroids tested, none was more potent than pregnanolone. Other potent inhibitors, with the exception of norethindrome, had a similar structure (C_{21}) with hydroxyl or keto groups at C_3 and C_{20}. Inhibitory activity was not related to the endocrine activity of the steroid. Inhibition by a group of 11 steroids was generally greater with 9000 *g* supernatants from female rats, and was uniformly increased with supernatants from pregnant rats. By 10 days after delivery, inhibition returned to levels equivalent to those found with supernatants from virgin females. Cholesterol and related compounds, fatty acids and esters, ceramides, gangliosides, phosphatidyl inositol and other natural fatty acid conjugates and a variety of indoles and lipids had no inhibitory activity. Pregnanolone administration increased the duration of hexobarbital- and of barbital-induced sleep in rats, apparently due to an additive sedative effect. However, microsomes prepared from these animals exhibited lesser rates of demethylation, with kinetics compatible with uncompetitive inhibition. These results indicate that progesterone, its metabolites and structurally similar steroids are potent inhibitors of microsomal drug metabolism. Such inhibition may be responsible in part for the low rates of drug metabolism seen in the female, during pregnancy, and in the neonate.

THAT STEROIDS may inhibit drug metabolism has been known for nearly a decade. Conney[1] summarized the evidence for the similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in hepatic microsomes. Indirect evidence indicates that steroid–drug interactions at a common site of biotransformation may be of physiological and pharmacological importance.

It is known that drug metabolism is decreased during pregnancy, as well as in women receiving the oral contraceptives.[2] Feuer and Liscio[3] and Rodriguez *et al.*[4] have shown that drug metabolism is decreased during pregnancy in rats, and that this decrease can be mimicked by administration of progesterone and progesterone-like steroids.[5] Moreover, studies in newborn rats indicated that early weaning increased the rate of maturation of drug-metabolizing capacity, whereas animals treated with progesterone metabolites continued to show low levels of drug-metabolizing activity.[6]

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Our previous studies have shown that several steroids which are probable metabolites of progesterone, particularly pregnanolane and close structural analogues, are potent inhibitors of model drug demethylation and hydroxylation reactions.[7] The present studies were designed to expand on these findings.

MATERIALS AND METHODS

Animals. Albino rats (Sprague–Dawley derived) were obtained from Locke Erikson Laboratories (Maywood, Ill.). In most studies, males (40–60 days of age) were used, but females of the same age were employed when specified in the results.

Chemicals. The steroids were purchased from Sigma Chemical Co. (St. Louis, Mo.) or Mann Research Laboratories (New York, N.Y.). Most other test compounds and *p*-nitroanisole (pNA) were obtained from Sigma. The pNA was sublimed before use. Pregnanolone-^{3}H was obtained from New England Nuclear Corp. (Boston, Mass.). Oxindole was obtained from the Aldrich Chemical Company (Milwaukee, Wis.). Several compounds were gifts, for which the authors are duly grateful. Other compounds were obtained as follows: 3β-Hydroxy-20-oxo-pregn-5-ene-16α-carbonitrile (PCN), Upjohn Co. (Kalamazoo, Mich.); 5β-pregnane-21-ol-3,20-dione, Searle Laboratories (Skokie, Ill.); ethynodiol diacetate, G. D. Searle & Company (San Juan, Puerto Rico); chlormadione acetate, Lilly Research Laboratories (Indianapolis, Ind.), and alphaxalone, Glaxco Research Ltd. (Fulmer, Buckinghamshire, England).

Preparation of the enzyme source. After the rats were decapitated, the livers were excised, rinsed, weighed and minced in ice-cold 1·15% KCl and 1-g portions homogenized in 6 g of cold isotonic sucrose (0·25 M, pH 7·7, containing 2·0 g EDTA and 2·42 g Tris/l). The homogenates were centrifuged at 9000 *g* for 40 min and the supernatant was used immediately or on the following day. Storage at 4° for 24 hr caused only a 10 per cent loss in activity and no change in the relative inhibitory activity of several steroids tested with fresh and 1-day-old supernatants.

Demethylation assay. The *O*-demethylation of *p*-nitroanisole (pNA) was assayed via the production of *p*-nitrophenol (pNP). Two ml of 9000 *g* supernatant was supplemented with 0·2 mM NADP, 2 mM glucose 6-phosphate and 5 mM $MgCl_2$ in a final volume of 5 ml; the pNA concentration was 0·6 mM, and the incubation at 37° was carried out for 10 min. The reaction was linear for at least 15 min. Activity related directly to tissue concentration. In later studies, especially with the newborn livers, the assay was proportionately scaled down to a 1–ml incubation volume without affecting the reaction rate or the per cent of inhibition by various test compounds. Flasks were incubated in duplicate.

The reaction was terminated by addition of 2 ml (or a proportionately smaller volume if the 1-ml total incubation was used) of trichloroacetic acid (10%) and chilling on ice for 30 min. Five ml of the supernatant was added to 1 ml of 1 N NaOH, shaken with 3 ml dichloromethane (to remove unreacted pNA) and recentrifuged. The pNP concentration was measured at 400 nm. According to the standard curve, 0·180 optical density units represented 100 nmoles pNP produced in 10 min.

The test compounds in a suitable solvent (usually chloroform or methanol) were added to the incubation flasks and the solvent allowed to evaporate prior to addition of the other components. Evaporated solvent blanks had no effect on the demethylation rate.

*Hexobarbital metabolism.*The methods of Cooper and Brodie[8] were used.

Studies in vivo. Sleep time was considered to be the duration of loss of the righting reflex. All experiments were carried out at the same time of day in a quiet room.

RESULTS

Inhibition studies. Pregnanolone, at a concentration of $1{\cdot}3 \times 10^{-5}$ M, inhibited demethylation activity by hepatic 9000 *g* supernatants from male rats by 36 per cent (Table 1). No other steroid was found to be a more potent inhibitor. Norethindrone, a synthetic progestational steroid employed in the oral contraceptive "pill", exhibited equivalent inhibitory activity. Norethynodrel, a compound similar to norethindrone except for the position of the double bond (5–10 vs 4–5) had only 65 per cent of the activity of norethindrone. This decrease in activity was similar to the difference noted between compounds having the double bond in the A ring vs those with the double bond in the B ring, e.g. progesterone vs pregnenolone.[7]

The 11-keto and 11-hydroxy derivatives of progesterone were inhibitory, though less potent than pregnanolone. Stilbestrol and mestranol were moderately inhibitory, in contrast to the natural estrogenic compounds, which were inactive. Certain other $C_{21}O_2$ steroids were also active, as previously noted.[7] A number of $C_{21}O_3$ compounds were also active but were less potent than the $C_{21}O_2$ steroids. Steroids having a double bond in the 5–6 position were much less active than those having a saturated A ring, in either the 5α or 5β configuration, or the Δ-4 unsaturated compounds, all of which had equivalent inhibitory activity.

The C_{18} and C_{19} compounds, generally having estrogenic or androgenic activity respectively, had little or no inhibitory activity.

The major adrenocortical steroid in the rat, corticosterone, was inhibitory, though somewhat less potent than pregnanolone, whereas other natural and synthetic corticosteroids had little or no activity. The sodium-retaining adrenal steroid, desoxycorticosterone, had an inhibitory activity equivalent to that of corticosterone.

The potent inducers of hepatic drug-metabolizing enzyme activity, namely PCN and spironolactone, were less than one-half as potent as pregnanolone.

Cholesterol and cholesterol esters were inactive as inhibitors, as were a variety of fatty acids and a triglyceride.

Phospholipids were surveyed by use of brain extracts prepared by the Folch-Pi method.[9] Type I and III, containing primarily phosphatidyl inositide and phosphatidyl serine respectively, were inhibitory, but only in amounts about 50 times higher than the concentration of steroids used. Similarly, a crude preparation of gangliosides exhibited inhibitory activity, but only when tested at a high concentration. Two cephalins and cerebroside preparation were inactive.

During a longitudinal investigation of urinary steroid profiles during sexual maturation, Gupta *et al.*[10] reported finding a "steroid-like substance" yielding many of the typical steroid reactions, which was later identified as oxindole. Although the relationships between oxindole and steroid hormones are unclear, it is known that cortisone administration affects tryptophan metabolism by increasing hepatic tryptophan pyrrolase activity. Moreover, it is known that liver microsomes hydroxylate indole-containing compounds. Thus, it was of interest to study oxindole and several precursors and products.

None of the indoles was significantly inhibitory when tested at $1{\cdot}3 \times 10^{-5}$ M. At 10^{-4} M, 5-hydroxyindole, indoxyl acetate, isatin, tryptamine and serotonin showed

TABLE 1. INHIBITION OF pNA DEMETHYLATION BY VARIOUS COMPOUNDS*

Compound	N	% Inhibition (mean ± S.E.M.)
I. Steroids		
A. $C_{18}O_2$, $C_{18}O_3$		
Stilbestrol	2	28
Estrone	2	12
Estradiol-17β, estriol, estriol-3-methyl ether, 19-nortestosterone	2–3	inactive
B. $C_{19}O$		
5α-Androstan-3-one	2	8
C. $C_{19}O_2$		
Etiocholan-17β-ol-3-one	2	31
Epi-androsterone	2	28
5β-Androstan-3,17-dione	6	25 ± 3
Androsterone	5	13 ± 1
5β-Androstan-3α,17β-diol	2	12
5α-Androstan-17β-ol-3-one	4	11 ± 1
Etiocholan-3β-ol-17-one	2	10
Androstadiendione, 5α-androstan-3β,17β-diol, 5β-androstan-3α-ol-17-one	3	inactive
D. $C_{20}O_2$		
Norethindrone	5	36 ± 2
Norethynodrel	7	24 ± 3
17-Ethynyl estradiol	2	22
17α-Methyl-androstan-17β-ol-3-one	2	17
17α-Methyl-testosterone	2	14
Ethinodiol diacetate	2	13
E. $C_{21}O_2$		
Progesterone	2	38
Pregnanolone	8	36 ± 4
Ethisterone	2	9
F. $C_{21}O_3$		
5β-Pregnane-21-ol-3,20-dione	3	35 ± 2
Pregn-4-ene-3,11,20-trione	7	27 ± 2
Pregn-4-ene-11α-ol-3,20-dione	6	25 ± 2
5α-Pregnane-3α-ol-11,20-dione (alphaxalone)	2	13
Pregn-5-ene-17α-ol-3,20-dione	4	inactive
G. Miscellaneous		
Pregn-4-ene-16α-methyl-3,20-dione	6	20 ± 4
Mestranol	4	20 ± 2
Chlormadione	3	17 ± 2
6α-Methyl-17α-hydroxy-progesterone	2	12
6α-Methyl-16-dehydro-progesterone	2	10
Pregn-5-ene-3β-ol-20-oxo-16α-carbonitrile (PCN)	4	9 ± 2
16β-Methyl-progesterone	2	6
Prednisolone	5	5 ± 1
Pregn-5-ene-3α,17α-diol-20-one	1	5
Pregn-5-ene-16β-methyl-3α-ol-20-one	1	4
Cortisol	2	0

TABLE 1. (*cont.*)

Compound	N	% Inhibition (mean ± S.E.M.)
II. Indoles and related compounds		
Indole, oxindole, 5-hydroxy-indole, indole-3-acetate, indoxyl acetate, isatin, anthranilic acid, DL-tryptophan, 5-hydroxy-DL-tryptophane, tryptamine, 5-hydroxytryptamine	2	0
Indoles active at $1{\cdot}3 \times 10^{-4}$ M		
Indoxyl acetate	3	33 ± 4
Tryptamine	3	20 ± 5
5-Hydroxyindole	2	16
Isatin	2	15
5-Hydroxytryptamine	3	12 ± 4
Oxindole	4	6 ± 0·4
III. Lipids		
DL-α-Cephalin, L-α-cephalin, cerebrosides, cholesterol, cholesterol *n*-butyrate, laurate and palmitate, *n*-butyric, palmitic, linoleic, and trilinoleic acid	3–4	inactive
IV. Brain extracts		
A. Folchi-Pi Type I† (1 mg)	4	20 ± 2
Type III‡ (1 mg)	4	16 ± 1
Type V§ (1 mg)	2	<5
Type VI‖ (1 mg)	2	<5
B. Sphingomyelin (10 μg)	2	0
C. Ceramides (200 μg)	2	0
D. Gangliosides (100 μg)	2	0
Gangliosides (500 μg)	4	12 ± 4
V. Miscellaneous		
SKF-525A	4	26 ± 4
c-AMP (10^{-8}–10^{-4} M)	2	0

* Inhibitor concentration was $1{\cdot}3 \times 10^{-5}$ M, except when stated in the table. N = number of individual determinations using homogenates from different pools of livers; each assay was performed in duplicate. Control activity was 120 ± 6 nmoles pNP produced in 10 min.

† Contains phosphatidyl inositide and other natural fatty acid conjugates.

‡ Contains phosphatidyl L-serine and other natural fatty acid conjugates.

§ Contains phosphatidyl ethanolamine and other natural fatty acid conjugates.

‖ Contains phosphatidyl ethanolamine, cerebrosides, cerebroside sulfates, sphingomyelin and gangliosides.

inhibitory activity (12–33 per cent), while anthranilic acid, indole-3-acetic acid, tryptophan and 5-hydroxytryptophan still showed less than 3 per cent inhibition. At 10^{-3} M, oxindole and indole exhibited 52 and 72 per cent inhibition respectively.

Cyclic AMP was inactive at the concentrations employed (up to 10^{-4} M). This is in agreement with the findings of Weiner *et al.*[11,12] that direct addition of cyclic nucleotides to whole liver homogenates, 9000 *g* supernatant fractions or microsomes did not inhibit drug metabolism, although inhibition was demonstrated by these authors in subfractions prepared from rats pretreated with dibutyryl cyclic 3′,5′-adenosine monophosphate or a glucagon–theophylline combination.

The classical inhibitor of drug metabolism, SKF-525A, was somewhat less potent than pregnanolone, though more potent when supernatants from females were tested (see below).

Threshold studies. Studies employing dilute concentrations of inhibitors, and assuming 5 per cent inhibition to be the practical threshold of the assay, revealed that at $1{\cdot}3 \times 10^{-8}$ M inhibition was found with pregnanolone, 11-keto pregesterone, norethindrone, mestranol and 21-hydroxypregnane-3,20-dione. At 10^{-9} and 10^{-10} M, inhibition could not be consistently detected.

Inhibition of hepatic supernatants from newborn, female and pregnant rats. Since endogenous steroid levels vary with age, sex and the female cycle, we compared the inhibition potency of selected compounds using 9000 *g* supernatants prepared from livers of female, pregnant, lactating and newborn rats. The demethylation activity varied as expected, being decreased in females, during pregnancy and in newborns (Table 2).

A comparison of the inhibitory activity of a variety of steroids on the enzyme activity in supernatants prepared from female rather than male rats did not reveal any consistent pattern. Several compounds exhibited marked differences, e.g. corticosteroid was apparently three times more potent with supernatants from females, whereas pregnanolone and androsterone were more potent inhibitors with supernatants from males (Table 2).

All compounds examined exhibited an increase in potency when tested against supernatants prepared from livers of pregnant rats. During lactation, the inhibition potency of all compounds returned to approximately the same level as that seen with the supernatants from virgin females.

With supernatants from 5-day-old rats, inhibition by pregnanolone and norethindrone was about one-half that seen with supernatants from males; that with andros-

TABLE 2. INHIBITION OF pNA DEMETHYLATION BY HEPATIC SUPERNATANTS AS A FUNCTION OF SEX AND PHYSIOLOGICAL STATE

	% Inhibition				
Compound	Adult male	Adult female	Pregnant (17–20 days)	Lactating female	Newborn
Pregnanolone	35 ± 2	22	36	21	19
Progesterone	38	31	41	30	
Corticosterone	14 ± 4	38	52	41	
Norethindrone	34	43	60	42	17
Androsterone	12	3	6	0	11
19–Nortestosterone	11 ± 3	19	27	15	25
Mestranol	7	14	37	20	11
Estradiol	12 ± 5	5	10	6	
Stilbestrol	20 ± 1	19	22	20	
Cholesterol	3 ± 3	3	13	9	
SKF-525A	26 ± 3	47	59	51	

* Values are the means of two to four experiments (± S.E.M. when N = 4). All compounds were tested at $1{\cdot}3 \times 10^{-5}$ M, except cholesterol which was 10^{-3} M. The lactating females were studied 10–14 days after delivery. Control activity (nmoles pNP produced in 10 min): males, 126; females, 86; pregnant, 77; lactating, 95; and newborn, 25.

terone and 19-nortestosterone was greater with supernatants from newborns, whereas the inhibitory activity of norethindrone and mestranol was greater with supernatants from their mothers.

The inhibitory potency of pregnanolone was about equal with supernatants from newborns, lactating and virgin females, and was 75 per cent higher in both males and pregnant females.

Two of the more active indoles (at 10^{-4} M in males) were even more potent against supernatants of livers from females (tryptamine, 48 vs 20 per cent; 5-hydroxytryptamine, 47 vs 12 per cent), while the degree of inhibition observed with 5-hydroxyindole and indoxyl acetate was identical with supernatants from both sexes.

Influence of pregnanolone on hexobarbital sleep time and metabolism. To determine if the inhibitory effect of pregnanolone was detectable *in vivo*, studies were undertaken employing hexobarbital. This drug had the advantage over pNA that methods were established for determining its rate of metabolism *in vitro*, and the duration of its pharmacologic effect, relating to its rate of metabolism *in vivo*, was easily defined by loss of the righting reflex (sleep time).

TABLE 3. INFLUENCE OF INTRADOSE INTERVAL ON PREGNANOLONE'S PROLONGATION OF HEXOBARBITAL SLEEP TIME

Pretreatment*	Time between drugs (min)	N	Sleep time (min)	P
Saline		11	26·7 ± 4·4	
Pregnanolone	0	4	22·5 ± 4·7	NS
Pregnanolone	30	6	42·2 ± 3·5	0·05
Pregnanolone	90	5	34·2 ± 4·2	NS
Pregnanolone	180	6	25·0 ± 6·0	NS

* A nonhypnotic dose of pregnanolone, 20 mg/kg i.p., was administered at various times before administration of hexobarbital, 75 mg/kg i.p.

Hexobarbital was administered at four times periods, from concurrent to 3 hr after pregnanolone. The dose of pregnanolone was about one-half the hypnotic dose and one-tenth the LD_{50} for weanling rats.[13] When hexobarbital was administered 30 min after pregnanolone, sleep time was significantly prolonged (Table 3). This finding was confirmed in a crossover study in which the same rat received saline or pregnanolone 30 min after i.v. hexobarbital. Five of the six rats clearly had longer sleeping times with pregnanolone pretreatment (matched pairs *t*-test, $P < 0{\cdot}025$).

Similar findings were obtained when hexobarbital was administered i.v. (rather than i.p.). When pregnanolone pretreatment was given 36 min before hexobarbital, the sleep time was 22 ± 3 min compared to a control of 9 ± 1 min ($P < 0{\cdot}05$), whereas with a 60-min pretreatment interval the sleep time was only 12 ± 3 min, not significantly different from that of controls.

The serum concentration of hexobarbital of animals killed upon awakening was lower in pregnanolone-pretreated rats (38·0 ± 3·0 vs 47·4 ± 3·5 mg/ml; $N = 10$, $P < 0{\cdot}05$). To differentiate further between inhibition of hepatic metabolism vs an additive or synergistic sedative effect at the central nervous sytem (CNS) level, the influence of pregnanolone on barbital, a nonmetabolized barbiturate, was examined. Onset of sleep was more rapid and duration was prolonged by pregnanolone pretreatment (Table 4).

TABLE 4. INFLUENCE OF CONCURRENT PREGNANOLONE ADMINISTRATION ON THE ONSET AND DURATION OF BARBITAL-INDUCED HYPNOSIS

Treatment*	N	Loss of righting reflex Onset (min)	Loss of righting reflex Duration (min)
Barbital	8	46·6 ± 3·1	92·5 ± 15·3
Pregnanolone and barbital	6	30·8 ± 7·5†	85·3 ± 6·5‡
Pregnanolone	6	0	

* Dose of sodium barbital was 150 mg/kg i.p.
† Differs from control ($P < 0.05$).
‡ Three rats did not recover by 4 hr and are not included. The longest duration in the control group was 136 min.

In addition, experiments were performed *in vitro* on the metabolism of hexobarbital with 9000 *g* supernatants obtained from rats injected with pregnanolone 30 min before sacrifice. The per cent disappearance of substrate was equivalent (control = 12·3 per cent vs pretreated = 13·3 per cent).

Kinetic studies of the rate of hexobarbital metabolism *in vitro* by 9000 *g* supernatants from control and pregnanolone-pretreated rats with four concentrations of substrate revealed no difference in V_{max} or K_m. These data were in contrast to those obtained with pNA demethylation (see below). Thus, the prolongation of hexobarbital sleeping time induced by pregnanolone pretreatment was not due solely to inhibition of hepatic metabolism.

Demethylation activity after pregnanolone administration. To examine the question whether the negative results obtained with hexobarbital reflected a difference between substrates, similar studies were performed with pNA. Rats were injected with the same dose of pregnanolone and killed 30 min later, together with saline-treated control animals. Demethylation activity of the 9000 *g* supernatant was significantly less

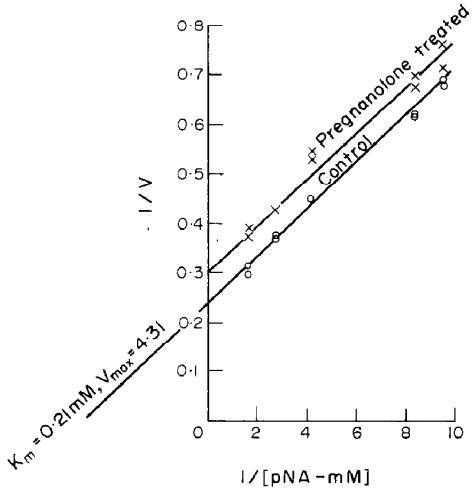

FIG. 1. Lineweaver–Burk plot of demethylation activity of 9000 *g* supernatants from pregnanolone-pretreated (×) and control (○) rats. V = nmoles pNP/mg of protein.

TABLE 5. INHIBITION OF DEMETHYLATION BY EXTRACTS OF MICROSOMES FROM CONTROL AND PREGNANOLONE-TREATED RATS

Amount of extract tested* (ml)	% Inhibition by extract microsomes	
	Control	Pretreated†
0·1		26
0·33		39
1·0	26	48
3·2	44	73

* One ml of extract was derived from about 20 mg of microsomal protein.

† The pretreated rats received 20 mg/kg i.p. of pregnanolone 30 min prior to sacrifice.

in the pregnanolone-pretreated animals (mean ± S.D. values of 1·7 ± 0·5 vs controls 2·2 ± 0·3 nmoles/mg protein; $P < 0·01$).

The plots ($1/V$ vs $1/S$) of kinetic studies employing five substrate concentrations (0·1 to 0·6 mM) with the same 9000 *g* supernatants exhibited parallelism (Fig. 1). This uncompetitive type of inhibition was found when pregnanolone was added directly to the incubation flask.[7]

Ether extracts of microsomes from pretreated rats had about twice the inhibitory activity of similar extracts from controls (Table 5). Thin-layer chromatography[14] revealed two prominent peaks, having R_f values coincident with authentic pregnanolone and 5β-pregnane-3α,20β-diol. The latter steroid has about one-third the inhibitory potency of pregnanolone when tested *in vitro*.[7]

DISCUSSION

The results of the present study, together with prevously published data,[7] indicate that about 18 pregnene and prenane steroids structurally similar to progesterone were potent inhibitors of a model microsomal drug-metabolizing enzyme system, the *O*-demethylation of pNA. This inhibitory activity was in contrast to the much lower inhibition exhibited by a wide variety of other steroids, some 60 in number, many of which presumably are natural or synthetic substrates for mixed-function oxidation

TABLE 6. INFLUENCE OF ADDITIONAL GROUPS ON THE INHIBITORY ACTIVITY OF PROGESTERONE*

	% Inhibition
Progesterone	38
Additional groups	
11-Keto	27
11-OH	25
17α-OH	21
16α-Me	20
6α-Me, 17α-OH	12
16β-Me	6
11β,17α,21-Triol (cortisol)	0

* As with the pregnanes, functional groups other than at C_3 and C_{20} decreased inhibitory potency, whereas endocrine activity tends to be enhanced by such additions.

reactions. Thus, potent inhibitory activity appears to be limited to steroids with a relatively characteristic structure.

The potent steroids, except norethindrone, were C_{21} having hydroxyl or keto groups at C_3 and C_{20}. Of these, six had further hydroxyl or keto substitutions at C_{11}, C_{17} or C_{21}, all of which tended to reduce inhibitory activity. This tendency can be seen with the series of progesterone derivatives shown in Table 6.

The Δ-5 compounds (B ring unsaturated) were definitely less active than the A ring (Δ-4) unsaturated steroids. For example, norethynodrel was significantly less active than norethindrone and pregnenolone was less potent than progesterone.

Saturation of the Δ-4 double bond of progesterone caused a decrease in inhibitory activity when in the α-position, but no change when in the β-position (Table 7). In general, the 5β pregnanes were more inhibitory than the 5α series. A high degree of stereospecificity was seen relative to the hydroxyl group at C_{20}, with the alpha steroids being inactive (Table 7). At C_3, the alpha hydroxyl or keto derivatives were more inhibitory than the β compounds.

TABLE 7. EFFECT ON INHIBITORY POTENCY OF PROGESTERONE BY SATURATION IN THE 5α AND 5β CONFIGURATIONS, AND THE INFLUENCE OF REDUCTION AT C_{20} IN THE α- AND β-POSITIONS ON THE INHIBITORY POTENCY OF EPI- AND ALLO-PREGNANEDIOLS

	% Inhibition
Saturation of A ring	
Progesterone	38
5α-Pregnane-3,20-dione	10
5β-Pregnane-3,20-dione	42
Reduction at C_{20}	
Pregnanolone	40
5β-pregnane-3α,20α-diol	0
5β-Pregnane-3α,20β-diol	12
Allo-pregnanolone	17
5α-Pregnane-3α,20α-diol	0
5α-Pregnane-3α,20β-diol	27
5α-Pregnane-3β,20β-diol	12

The present studies *in vivo* following pregnanolone administration indicate effects on brain and liver. The former was shown by an additive effect on the duration of sleep after concurrent administration of the nonmetabolized barbiturate, barbital, and by lower serum levels of hexobarbital upon awaking. Effects of inhibition by pregnanolone or a metabolite(s) could be shown on pNA metabolism with microsomes harvested from pretreated animals.

The results of these studies, coupled with data in the literature,[3,5,6] lead to the proposal that inhibition of the hepatic mixed-function oxidase system by pregnane steroids may play a physiologic role in the intact organism. High levels of progesterone and its metabolites during pregnancy, and probably during the immediate newborn period, as well as in the nonpregnant female as compared to the male, may explain the relatively low rates of drug metabolism in these states.

If this proposal is correct, should be possible to extract such steroids from the microsomes. During the past year, we have prepared lipid extracts of microsomes from pregnant, newborn and adult animals, and have been able to show that such

extracts inhibit pNA demethylation.[15] Moreover, thin-layer chromatography of these extracts have shown that the most potent inhibitory materials migrate with R_f values comparable to those of the pregnane inhibitors. The negative results obtained with lipids and other membrane components in the present study are thus of considerable importance to the extraction studies, for they infer that the extracted inhibitors are probably not substances which are part of the structural lipoprotein of the endoplasmic reticulum. Studies are in progress to define further these inhibitory substances.

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