Effects of Various Drugs on the Uterotropic Response to Mestranol and Norethynodrel in the Rat (35189)

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Mestranol and norethynodrel are synthetic steroids commonly used in oral contraceptive drug formulations. The efficacy of such preparations may be affected by other drugs which influence the hormonal activity of these agents.

The present study utilizes the induction phase response of the rat uterus to a single dose of hormone as a method of evaluating possible interactions between mestranol, norethynodrel, and various other drugs.

Materials and Methods. Immature female rats (19 days old) were obtained from the Holtzman Colony, Madison, Wisconsin. The rats were caged individually and received Purina laboratory chow and water *ad libitum*. Animal quarters were kept at a constant temperature (24°) and on a controlled constant lighting schedule (12 hr dark, 12 hr light).

Drugs were dissolved in saline with the exception of phenylbutazone and diphenylhydantoin, which were suspended in carboxymethylcellulose medium. Drugs were administered intraperitoneally twice daily for 3 days at the following doses: phenobarbital, 37 mg/kg; SKF 525A (Proadifen) 40 mg/kg; tolbutamide, 125 mg/kg; chlorpromazine, 5 mg/kg; imipramine, 8 mg/kg; para-aminosalicylic acid, 3 mg/kg; diphenylhydantoin, 125 mg/kg; phenylbutazone, 60 mg/kg; scopolamine hydrobromide, 0.5 mg/kg. The total volume per dose was 0.2 ml.

Mestranol and norethynodrel were dissolved in corn oil and administered in a volume of 0.2 ml by oral intubation 18 hr following the last pretreatment dose. Saline was used as the control pretreatment injection and corn oil as the control for the steroids.

Four hr after oral administration of the steroid, the rats were weighed, and sacrificed by cervical dislocation. Their uteri were excised, trimmed of extraneous tissue, and weighed on a Roller Smith torsion balance.

TABLE I. Effects of Phenobarbital and SKF 525A on the Uterotropic Response to Mestranol[a] in the Rat.

Pretreatment	Treatment	No. of females	Uterine wt/ body wt (mg/g)
Saline	Corn oil	27	0.51 ± 0.01[b]
Phenobarbital[c]	Corn oil	10	0.43 ± 0.01—[e]
SKF 525A[d]	Corn oil	17	0.62 ± 0.01+
Saline	Mestranol	28	0.66 ± 0.00
Phenobarbital	Mestranol	16	0.52 ± 0.01—
SKF 525A	Mestranol	17	0.85 ± 0.01+

[a] Mestranol: 1 μg/animal, single injection.

[b] Indicates ± standard error of the mean.

[c] Phenobarbital was administered at 37 mg/kg ip twice daily for 3 days.

[d] SKF 525A was administered at 40 mg/kg ip twice daily for 3 days.

[e] The symbols (+), (—) indicate significant ($p < 0.001$) increases or decreases from saline pretreatment.

Because variability in the body weights of immature rats is reflected in their uterine weights, the uterine wet weights were calculated as milligrams of uterine tissue per gram of body weight.

Results. The effect of pretreatment with phenobarbital or SKF 525A on the uterine wet weight response after an oral dose of mestranol are shown in Table I. Phenobarbital significantly decreased uterine wet weight in control animals as well as in those treated with 1 or 3 μg of mestranol. On the other hand, pretreatment with SKF 525A significantly increased the uterine wet weight in control and mestranol-treated groups.

TABLE II. Effects of Various Drugs on the Uterotropic Response to Mestranol in the Rat.

Pretreatment compounds[a]	Dose (mg/kg)	Corn oil		Mestranol, 1 μg	
		No. of females	Uterine wt/body wt (mg/g)	No. of females	Uterine wt/body wt (mg/g)
Saline	—	23	0.48 ± 0.00[b]	31	0.67 ± 0.00
Chlorpromazine	5	8	0.49 ± 0.01	13	0.80 ± 0.02+[c]
Imipramine	8	15	0.46 ± 0.01	19	0.76 ± 0.02+
para-Aminosalicylic acid	3000	8	0.48 ± 0.02	20	0.74 ± 0.01+
Tolbutamide	125	8	0.55 ± 0.01+	10	0.78 ± 0.02+
Diphenylhydantoin	125	7	0.45 ± 0.02	10	0.55 ± 0.01—
Scopolamine • HBr	0.5	8	0.50 ± 0.01	8	0.61 ± 0.01—
Phenylbutazone	60	8	0.48 ± 0.01	8	0.61 ± 0.01—

[a] Each compound was administered twice daily ip for three days.

[b] Indicates mean ± standard error of the mean.

[c] The symbols (+), (—) indicate significant increases and decreases ($p<0.01$) from saline pretreatment.

Table II shows alterations in uterine wet weight response to 1 μg mestranol following pretreatment with various drugs. Only tolbutamide was found to increase the uterine wet weight in controls. Compounds found to significantly increase the response to 1 μg of mestranol were chlorpromazine, imipramine, *para*-aminosalicylic acid (PAS) and tolbutamide. Significant decreases in the response to 1 μg of mestranol were found following pretreatment with diphenylhydantoin, scopolamine hydrobromide, and phenylbutazone.

Drug-induced alterations in the uterine wet weight response to norethynodrel are shown in Table III. Following pretreatment with phenobarbital or diphenylhydantoin the response to 4 μg of norethynodrel was abolished. Pretreatment with SKF 525A resulted in significant increases in response over norethynodrel controls.

Discussion. The response of the uterus to a single dose of estrogen can be divided into three phases: induction, RNA accumulation, and DNA synthesis (1). The early response (induction phase) reaches its peak at 4–6 hours and is characterized by generalized hyperemia and an accumulation of fluid resulting in increased uterine wet weight. At the peak of the early response, histological sections reveal increased spacing between nuclei and an increase in cell size. During this phase there is little or no change in uterine RNA, DNA, or protein.

Of the drugs tested, only tolbutamide produced an increase in both the uterine wet weight of the control group and in the induc-

TABLE III. Effects of Various Drugs on the Uterotropic Response to Norethynodrel[a] in the Rat.

Pretreatment compound[b]	Dose (mg/kg)	Treatment compound	No. of females	Uterine wt/body wt (mg/g)
Saline	—	Corn oil	10	0.49 ± 0.01[c]
	—	Norethynodrel	19	0.57 ± 0.01
Phenobarbital	37	Norethynodrel	16	0.49 ± 0.02—[d]
Diphenylhydantoin	125	Norethynodrel	8	0.47 ± 0.02—
SKF 525A	40	Norethynodrel	9	0.79 ± 0.03+

[a] Norethynodrel: 4 μg/animal, single injection.

[b] Pretreatment compounds were administered twice daily ip for three days.

[c] Indicates mean ± standard error of the mean.

[d] The symbols (+), (—) indicate significant ($p<0.02$) increases and decreases from control.

tion phase response to mestranol. Direct estrogenic action by drugs has been demonstrated in immature and mature ovariectomized rats. Welch *et al.* (2) demonstrated the estrogenic potency of DDT and its analogs and Saad (3) described a slight increase in uterine weight following the administration of tolbutamide to ovariectomized young rats. The estrogen-like activity of tolbutamide was probably unrelated to its hypoglycemic action since the increase in uterine weight was not observed with acetohexamide.

In the present study, treatment of immature female rats with phenobarbital reduced the response to mestranol or norethynodrel given orally. Similar results have been reported by Levin *et al.* (4). Several studies have shown the chronic treatment of animals with drugs or pesticides (5, 6) induces hepatic microsomal enzymes and accelerates the metabolism of estradiol-17β or estrone to polar metabolites. Fahim *et al.* (7) has also reported that treatment with phenobarbital causes a decrease in the uterine wet weight of intact mature mice. Conversely, pretreatment with SKF 525A, an inhibitor of steroid hydroxylase in hepatic microsomes (8), increased uterine wet weight of controls and increased the uterotropic response to mestranol and norethynodrel.

Para-Aminosalicylic acid (PAS) also significantly increased the uterotropic action of mestranol. Although Rogers *et al.* (9) reported that PAS induced prolongation of hexobarbital sleeping time by inhibition of the microsomal enzyme system, a mechanism of action different from that of SKF 525A was suggested since PAS does not inhibit hexobarbital metabolism until several days after administration. Also, this decreased activity of microsomal enzymes was accompanied by an increase in hepatic glycogen in contrast to the decrease usually seen with SKF 525A.

Pretreatment with chlorpromazine or imipramine increased the uterotropic response to mestranol. Stitzel *et al.* (10) found chlorpromazine to be a potent inhibitor of the hexobarbital-metabolizing enzyme systems in the perfused rat liver. In addition, Tolson *et al.* (11) reported that treatment with chlorpromazime or imipramine potentiated cortisol induction of hepatic tyrosine transaminase activity. Kato *et al.* (12) observed that imipramine exhibited a strong inhibitory action on the *in vivo* and *in vitro* metabolism of barbiturates, carisoprodol, and strychnine.

Pretreatment with diphenylhydantoin or phenylbutazone decreased the uterotropic response to mestranol as did phenobarbital. A decrease in the uterine wet weight response to norethynodrel following pretreatment with diphenylhydantoin was also observed. Similarly, Conney *et al.* (13) reported a marked increase in the 6β-hydroxylation of cortisol by hepatic microsomal enzymes after guinea pigs were treated with diphenylhydantoin and also observed stimulation of enzymatic hydroxylation of testosterone following pretreatment with phenylbutazone (7).

In studies by Barnea (14), chemical blockade of the sympathetic nervous system by pretreatment with reserpine or syrosingopine decreased uterine wet weight response to estradiol-17β and estradiol benzoate in immature rats; whereas pretreatment with guanethidine, a more peripheral suppressor of sympathetic transmission, was ineffective. In the present study, a decrease in the uterotropic response to mestranol was found following pretreatment with scopolamine hydrobromide, a peripheral antimuscarinic compound similar to atropine. Thus, blockade of uterine innervation could account for these findings.

Drug-induced alterations in the uterotropic potency of the two steroids used in these experiments may occur by at least three different mechanisms: direct estrogenic activity, changes in the activity of drug metabolizing enzymes, and blockade of uterine innervation. Uterine wet weight response in immature rats is a sensitive and simple method for evaluating possible drug interactions with steroids used in clinical drug therapy.

Summary. Alterations in the uterine wet weight response to mestranol or norethynodrel were found to occur following pretreatment of immature rats with various drugs. Pretreatment with phenobarbital or SKF 525A was found to decrease and increase, respectively, the uterine wet weight in con-

trols as well as in mestranol-treated groups. Of the other drugs tested, only tolbutamide increased uterine wet weights in both control and mestranol-treated groups. In addition, the response to mestranol was increased following pretreatment with *p*-aminosalicylic acid, chlorpromazine, and imipramine and decreased following diphenylhydantoin, phenylbutazone, or scopolamine pretreatment. The response to norethynodrel was decreased after pretreatment with phenobarbital or diphenylhydantoin and increased following SKF 525A pretreatment. The test system used in these experiments was sensitive to drugs acting by different mechanisms and therefore could prove useful in predicting interaction between drugs and steroids used in treating gynecologic problems.

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1. Mueller, G. C., Herranen, A. M., and Jervell, K. F., Recent Progr. Horm. Res. **14**, 95 (1958).

2. Welch, R. M., Levin, W., and Conney, A. H. Toxicol. Appl. Pharmacol. **14**, 358 (1969).

3. Saad, S. F., J. Pharm. Pharmacol. **21**, 314 (1969).

4. Levin, W., Welch, R. M., and Conney, A. H., Endocrinology **83**, 149 (1968).

5. Welch, R. M., Levin, W., and Conney, A. H., J. Pharmacol. Exp. Ther. **160**, 171 (1968).

6. Levin, W., Welch, R. M., and Conney, A. H., J. Pharmacol. Exp. Ther. **159**, 362 (1968).

7. Fahim, M. S., King, T. M., and Hall, D. G., Amer. J. Obstet. Gynecol. **100**, 171 (1968).

8. Conney, A. H., Schneidman, K., Jacobson, M., and Kuntzman, R., Ann. N. Y. Acad. Sci. **123**, 98 (1965).

9. Rogers, L. A., Alcantara, G. A., and Fouts, J. R., J. Pharmacol. Exp. Ther. **142**, 242 (1963).

10. Stitzel, R. E., Tephly, T. R., and Mannering, G. J., Mol. Pharmacol. **4**, 15 (1968).

11. Tolson, W. W., Colson, S., and Umberger, E., Life Sci. **8**, 1175 (1969).

12. Kato, R., Chiesara, E., and Vassanelli, P., Biochem. Pharmacol. **13**, 69 (1964).

13. Conney, A. H., Jacobson, M., Schneidman, K., and Kuntzman, R., Life Sci. **4**, 1091 (1965).

14. Barnea, A., Endocrinology **84**, 746 (1969).

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