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Received October 26, 1959. P.S.E.B.M., 1960, v103.

A-Norprogesterone, an Androgen Antagonist. (25448)

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Typical growth responses of accessory sex organs of the rat or the chick comb to androgen have been inhibited by several substances. These include progesterone(1,2), estrone(2, 3), estradiol(2,4), dehydroepiandrosterone (5), methylcholanthrene(6), 2-acetyl-7-oxo-1, 2,3,4,4a,4b,5,6,7,9,10,10a - dodecahydrophenanthrene(7,8), and 20-methyl-Δ^5-pregnene-3β,20-diol(9); all being steroids or steroid-like in configuration. Structural resemblance of such compounds to natural steroids could form a basis for hormonal antagonism. Recently a series of A-norsteroids was synthesized by Weisenborn and Applegate(10). We studied these steroidal homologs for hormonal and anti-hormonal activities. This report is concerned with A-norprogesterone, which possesses anti-androgenic properties.

Material and methods. Androgenic and anti-androgenic activities. Twenty-three day old male rats (Sprague Dawley strain) weighing approximately 50-60 g were castrated and divided into groups of 4 or 6 animals. Test compounds were dissolved or suspended in sesame oil. A-norprogesterone alone and in combination with testosterone propionate was administered subcutaneously once daily for 7 days starting on day of castration. Animals were sacrificed on day following last injection, and the ventral prostate, seminal vesicles, coagulating gland, levator ani muscle, adrenals, thymus and thyroids were removed and weighed. Intact immature male rats weighing approximately 100 g were subjected to experimental technics similar to those described for the castrated animal. In addition the pituitary, testis, spleen, kidney, heart and liver were removed and weighed. Two day old White Leghorn cockerels, in groups of 24, were injected subcutaneously with a single .5 mg dose of testosterone enanthate (Delatestryl) in sesame oil as in the method described by Dorfman(8). A-norprogesterone was dissolved in sesame oil in several concentrations, and .05 ml was applied on the comb once daily for 7 days starting on day of androgen treatment. The chicks were sacrificed on day following last inunction and the comb and body weights were recorded. *Uterotrophic and anti-estrogenic activities.* Immature female mice of the Swiss Albino strain weighing approximately 7 g were divided into groups of 5 animals. A-norprogesterone dissolved in sesame oil was administered subcutaneously once daily for 3 days, either alone or in combination with a daily dose of .033 μg of estradiol benzoate. Animals were sacrificed on day following last injection. The quantity of uterine luminal fluid was estimated. This fluid was then expressed and the uteri were weighed. *Progestational and anti-progestational activity.* Immature female rabbits in groups of 4 animals were employed in a modification of the McPhail method(11). The animals were given 3 intramuscular injections of 5 μg of estradiol benzoate on days 1, 3, and 5. From sixth day on, they received 5 daily intramuscular injections of progesterone, A-norprogesterone or both compounds. Animals were sacrificed on day following last injection

TABLE I. Anti-androgenic Activity of A-norprogesterone in Immature Castrate Male Rats Treated for 7 Days.

Treatment & daily dose	No. animals	Initial body wt	Final body wt	Ventral prostate	Seminal vesicles & coagulating gland	Levator ani	Adrenals	Thymus	Thyroid
		g		mg					
Sesame oil	42	58.2 ± .5*	95.4 ± .7	11.3 ± .3	11.2 ± .3	21.6 ± .7	31.9 ± .6	410.2 ± 8.7	10.0 ± .3
Testosterone propionate (T.P.), 25 μg	42	61.8 ± 1.7	97.6 ± 1.1	58.1 ± 1.8	52.7 ± 1.7	31.2 ± .9	31.3 ± .6	362.7 ± 12.2	10.5 ± .3
A-norprogesterone (25 mg) + T.P. (25 μg)	3	57.0 ± .6	80.3 ± 4.3	13.1 ± 2.3	13.1 ± .5	17.3 ± 1.6	26.9 ± 1.6	272.7 ± 24.8	8.7 ± .7
A-norprogesterone (5 mg) + T.P. (25 μg)	4	57.5 ± 2.2	97.5 ± 3.9	31.9 ± 1.7	24.6 ± 1.2	20.3 ± 1.3	28.4 ± 1.3	352.6 ± 17.2	9.1 ± .5
A-norprogesterone (1 mg) + T.P. (25 μg)	6	53.2 ± 1.5	97.5 ± 4.0	36.2 ± 1.7	38.6 ± 3.6	29.0 ± 2.0	31.7 ± 1.5	340.2 ± 52.4	9.5 ± .4
A-norprogesterone (5 mg)	4	53.0 ± 1.3	89.5 ± 2.5	12.6 ± 1.5	8.6 ± .2	18.9 ± 1.4	29.3 ± .5	292.5 ± 48.4	9.1 ± .5
" (1 ")	6	55.5 ± 3.9	98.0 ± 2.9	11.6 ± .8	11.1 ± .7	22.3 ± 2.3	32.0 ± 1.3	383.7 ± 44.7	9.4 ± .2

* Stand. error of mean.

and the uteri weighed and graded histologically for glandular development.

Results. The anti-androgenic activity of A-norprogesterone in the androgen treated castrated immature rat is summarized in Table I. The androgen-induced hypertrophy of the ventral prostate was reduced with 1, 5 and 25 mg daily doses of A-norprogesterone by 47, 56 and 96%, respectively. Similarly these doses reduced the hypertrophy of seminal vesicles and coagulating gland by 34, 68 and 95%, respectively. Daily doses of less than 1 mg produced little or no significant changes in accessory sex organ weights of testosterone propionate treated animals. Levator ani weights were decreased to non-androgen treated control levels with 5 mg of this compound. The 25 mg dose of this A-norsteroid slightly reduced body weight gain and levator ani, adrenal, thyroid and thymus weights of the androgen-treated castrate rat. A-norprogesterone in daily doses of 5 mg did not alter ventral prostate weights in non-androgen treated castrates. It did, however, slightly reduce thymus, adrenal, levator ani and seminal vesicle and coagulating gland weights. Preliminary data have indicated that in this assay the oral activity of this substance is of a lower order than is the parenteral activity.

Subcutaneous administration of 25 mg of A-norprogesterone daily for 7 days to 100 g intact male rats resulted in 26% decrease in ventral prostate weight, a 46% decrease in seminal vesicle weight but no significant reduction in coagulating gland or levator ani weights (Table II). The adrenal and thyroid weights were slightly decreased. The thymus, pituitary, testis, spleen, heart, kidney and liver weights were unchanged.

A - norprogesterone antagonized comb growth effect of injected testosterone enanthate as well as comb growth of non-androgen-treated cockerels (Fig. 1). A single injection of the androgen increased the absolute comb weight by 100%, and the comb to body weight ratio by a similar percentage. This comb growth stimulation was completely prevented by total dose of 5 mg of A-norprogesterone. Doses of .04, .2 and 1.0 mg of this

TABLE II. Effect of 7-Day Treatment with A-norprogesterone in Intact Male Rats.

Treatment	Daily dose, mg	Initial body wt (g)	Final body wt (g)	Ventral prostate (mg)	Seminal vesicles (mg)	Coagulating gland (mg)
Sesame oil control		101.0 ± .6*	138.3 ± 2.7	62.8 ± 2.6	34.6 ± .5	10.0 ± .6
A-norprogesterone	25	97.5 ± .9	130.8 ± 1.7	46.2 ± 9.7	18.6 ± 3.0	9.0 ± .9

Treatment	Daily dose, mg	Levator ani (mg)	Adrenals (mg)	Thymus (mg)	Thyroid (mg)
Sesame oil control		32.8 ± 3.5	35.4 ± 1.2	370.7 ± 27.1	12.7 ±.4
A-norprogesterone	25	30.4 ± 2.1	26.3 ± .7	363.9 ± 2.4	9.5 ±.6

* Stand. error of mean.

compound suppressed androgen stimulated comb growth by 24, 41 and 78%, respectively. When administered to non-androgen treated birds, comb weight was reduced by 18%. Body weight gain was unaltered by any of the treatments.

Uterine weights of immature female mice were not increased after administration of 1 mg total doses of A-norprogesterone. This level of compound was unable to alter significantly the uterine hypertrophy induced by estradiol benzoate. A-norprogesterone, therefore, is not estrogenic or anti-estrogenic at this dose.

Intramuscular administration of a total dose of 5 mg of A-norprogesterone failed to produce any progestational effects in estrogen primed immature rabbits. The same dose of this A-norsteroid did not antagonize the progestational activity of 1 mg of progesterone in these primed animals.

Discussion. Results of studies in rat and chick indicate that A-norprogesterone possesses anti-androgenic properties. This activity is not limited to inhibition of exogenous androgen but also manifests itself against endogenous androgen, since in non-androgen treated animals the compound induces a decrease in weight of rat accessory sex organs and of chick combs. It has been reported that reduction in ventral prostate and seminal vesicle weights can be obtained by pituitary gonadotrophin inhibition such as that elicited by estrogen administration(12). On the other hand our results in castrate rats treated with androgen show that inhibition of accessory sex organ hypertrophy by this A-norsteroid is not dependent upon an anti-gonadotrophin effect. A-norprogesterone, at dose levels employed, was devoid of androgenic, estrogenic, anti-estrogenic, progestational and anti-progestational activities. The androgen antagonism, therefore, does not appear to relate to known hormonal effects. The decrease or absence of end organ growth response cannot be readily attributed to any general toxic effect of the compound, since the inhibition was seen at dose levels that caused no significant changes in body weights. It would appear, therefore, that A-norprogesterone competes with androgen at the end organ sites.

Summary. A-norprogesterone antagonizes testosterone induced accessory sex organ hypertrophy in the immature castrate male rat and comb growth stimulation in the chick. Growth of the ventral prostate, seminal vesicles, adrenals and thyroids of intact rats and

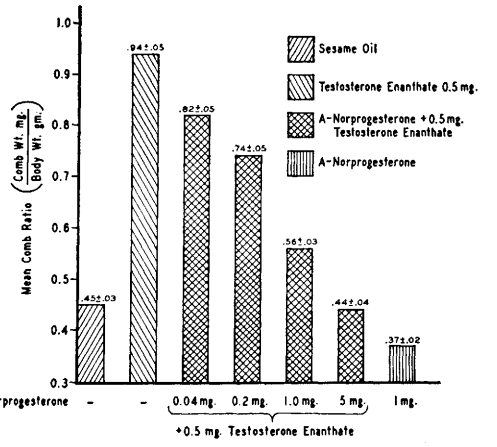

FIG. 1. Anti-androgenic activity as measured by inhibition of androgen-stimulated chick comb growth. Each bar represents the mean comb ratio ± stand. error of the mean. Initial body weights, 36.8 g to 38.7 g; final body weights, 81.7 g to 85.2 g (24 chicks/group).

the chick comb in animals not treated with androgen were significnatly reduced by administration of this compound. A-norprogesterone is not androgenic, estrogenic, anti-estrogenic, progestational or anti-progestational under the experimental conditions described.

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Received October 27, 1959. P.S.E.B.M., 1960, v103.

Biological Activities of Some 6-Methylated Progesterones.* (25449)

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Biological studies with a growing list of synthetic steroids have revealed marked increases in progestational potency that accompanied introduction of a 6α-methyl group into the molecule(1,2,3,4,5,6). Recent reports have indicated that adrenal depression is encountered in rats treated with some of these agents(7,8) and an earlier report from this laboratory showed that a relationship existed between progestational and estrogen-antagonistic potencies of relatives of 19-nortestosterone(9). The present report compares the progestational, estrogen - antagonistic and adrenal depressant properties of a series of 6-methylated derivatives of progesterone.

Materials and methods. Progestational activity was determined using our modification of uterine carbonic anhydrase assay(6) and the intrauterine assay of McGinty(10). Where possible, potency estimates for uterine carbonic anhydrase test were obtained from dose-response curves by comparing doses of compounds that produced approximately 50-60 enzyme units/100 mg tissue. Progesterone, administered subcutaneously in daily dose of 0.1 mg, produced this response and was used as the standard of comparison for compounds administered by either buccal or parenteral routes. Antagonism of estrone-induced uterine growth was determined in intact immature mice according to previously described methods(9). Briefly, estrone at 0.3 μg, which produced uterine weights averaging about 35 mg in controls, was mixed with test compounds. Estrogen antagonistic potency was determined from the dose of antagonist estimated to produce a 50% block of growth increment (oil controls averaged about 10 mg). Adrenal depressant activity was determined in spayed female rats. The animals were spayed at 30 days of age and received the test compound for 14 days, beginning 10 days after spaying (8).

Results. The metrotropic effects of various steroids are summarized in Table I. Introduction of 6α-methyl group into progesterone or 17α-acetoxyprogesterone was generally accompanied by increases in potency in each of the metrotropic assays. 6α-Methylprogesterone, which showed a slight decrease in potency in the estrogen antagonism test, was an

* Synthetic steroids used were prepared by Drs. P. B. Sollman, C. G. Bergstrom and W. M. Hoehn of the Division of Chemical Research, G. D. Searle and Co. and Dr. B. Löken, formerly of Root Chemical Inc., Roosevelt, Puerto Rico.