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# The Influence of Progesterone on Adrenocortical Function in the Rat[1] (38108)

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Estradiol and testosterone have differing influences on adrenal function. Estradiol augments adrenal corticosterone secretion by increasing ACTH secretion (1) and decreasing intraadrenal reduction of corticosterone through inhibition of adrenal 5α-reductase activity (2). Estradiol also stimulates hepatic metabolism and clearance of corticosterone (3). In contrast, testosterone inhibits ACTH secretion (4), but also facilitates adrenal corticosterone secretion through inhibition of adrenal 5α-reductase activity (2). Hepatic metabolism of corticosterone is suppressed by testosterone (5). These differences in pituitary, adrenal, and hepatic responses to testosterone and estrogen probably account for the sex differences in adrenal function seen in the rat (6).

The effects of progesterone on adrenal function are not as well understood as those of the other gonadal hormones. Several authors have reported that progesterone decreases adrenal corticosterone secretion (7) and adrenal weight (7, 8) when administered to female rats in doses exceeding 5 mg/day. Plasma corticosterone after ACTH treatment has been reported to be diminished after treatment with doses as low as 2.0 mg/day (9). In contrast, Telegdy *et al.* have shown that progesterone administered at a dose of 1.5 or 1.0 mg/day increases adrenal corticosterone secretion (7, 10) and adrenal weight (7). Ultrastructural changes in the fasciculata, reticularis, and glomerulosa of the adrenal cortex of female animals treated with progesterone (1.0 mg/day) have been interpreted by Volk (11) to indicate hyperactivity of these structures. Givner and Rochefort (12) have recently reviewed and extended the data concerning the effects of synthetic progestogens on adrenal function in female rats. The most intensively studied of these, 6 alpha-methyl-pregn-4-en-3, 20 dione-17-acetate (medroxyprogesterone), has usually been reported to produce adrenal atrophy, decreased plasma corticosterone concentration, and decreased *in vitro* corticosterone production. These effects have been ascribed in part to a block of pituitary ACTH secretion. Studies of the effect of progestogens on the adrenal function of ovariectomized animals are relatively few and contradictory. Edgren *et al.* (13) have reported that progesterone or 6-methyl-17 acetoxyprogesterone administration at a dose of 1 mg/day caused a decrease in adrenal weight of spayed females. In another experiment, only the latter preparation was effective. Brennen and Kray (14) found no significant effect of progesterone on adrenal weight when administered at either 1.0 or 3.0 mg/day to ovariectomized rats. The limited evidence concerning progesterone effects on male adrenal function indicates that injection of 10 mg/day results in decreased adrenal corticosterone secretion (9, 15) without a significant change in adrenal weight (15). In contrast, doses ranging from 0.3 mg to 3 mg/day caused increased adrenal weight (14).

The experiments reported here were undertaken to determine if progesterone exerts an influence on adrenal function in female rats independent of its attenuation of estrogen secretion (16). In addition, the effect of progesterone on adrenal 5α-reductase activity

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was assessed to determine if progesterone exercises an influence on this enzyme similar to that shown by the other gonadal hormones.

*Materials and Methods.* Female rats (body wt 175–200 g) of the Long–Evans strain, obtained from Blue Spruce Farms, Inc., Altamont, NY were used in all experiments. Animals were maintained under standardized conditions of light (0600–1800), and temperature (22.0 ± 0.5), on a diet consisting of Purina Laboratory Pellets and water ad lib. In the first experiment, 20 animals were divided randomly into two groups. Each rat in the first group received four subcutaneous polydimethylsiloxane capsules containing progesterone. They were implanted under ether anesthesia just anterior and parallel to each hind limb and just posterior and parallel to each fore limb. The capsules were similar to those described by Kincl and Rudel (17) and others (18, 19) and consisted of Silastic Medical Grade tubing (Dow Corning Corp., Midland, Mich., No. 602-231) filled with tightly packed dry crystalline progesterone to form a column 20 mm long. Capsules were sealed at each end with 2–3 mm of Dow Corning Medical Adhesive Type A. The animals in the control group received four empty capsules implanted similarly.

In the second experiment, 28 animals were ovariectomized and assigned to one of four equally sized groups in a 2 × 2 factorial design. At the time of operation, two groups received a single subcutaneous injection (10 μg/100 g body wt) of estradiolcyclopentylpropionate (Depoestradiol, Upjohn) and two received a control injection of oil solvent. Thirteen days later, four progesterone capsules identical to those described above were implanted in each member of one oil-treated and one estrogen-treated group. Empty capsules were implanted in the remaining groups. The animals in both experiments were sacrificed at 0800, 10 days after capsule implantation. Adrenal glands, uteri, trunk plasma, and progesterone capsules were collected at this time.

TABLE I. Effects of Progesterone in Intact Female Rats.[a]

| Measurement | Treatments | |
|---|---|---|
| | Control | Progesterone |
| Body wt (g) | 220 ± 8 | 239 ± 6 |
| Capsule wt loss (mg/10 day) | – | 20.2 ± 0.5 |
| Plasma progesterone (μg/100 ml) | 0.8 ± 0.2 | 5.4 ± 0.6[e] |
| Adrenal wt (mg) | 68 ± 2 | 58 ± 2[c] |
| Plasma corticosterone (μg/100 ml) | 22.8 ± 4.0 | 11.0± 3.7[c] |
| Homogenate corticosterone production (μg/10 mg/hr) | 4.2 ± 0.3 | 2.6 ± 0.3[d] |
| Homogenate total corticosteroid production (μg/10 mg/hr) | 6.0 ± 0.3 | 4.5 ± 0.3[d] |
| Reductase activity[b] | 14.4 ± 0.2 | 13.8 ± 0.5 |
| Uterine wt (mg) | 323 ± 18 | 270 ± 17[c] |

[a] Results are shown as means and standard errors of the mean. All values are based on 10 subjects per group except total corticosteroid production which is based on nine subjects per group.

[b] Expressed as μg corticosterone recovered/beaker, 12.5 μg having been added at the outset of incubation.

[c] $P < 0.05$.

[d] $P < 0.01$.

[e] $P < 0.001$.

Progesterone capsules were desiccated for at least 5 days prior to weighing, and an estimate of output was made from preimplantation and postsacrifice differences in capsule weight. Progesterone from plasma was separated from corticosterone with a Bush A paper chromatography system (20). Both progesterone and corticosterone were measured by competitive protein-binding techniques (21).

*In vitro* corticosterone production and adrenal 5$\alpha$-reductase activity were assayed in whole homogenates as previously described (22). Adrenal glands were homogenized in 0.25 *M* sucrose, supplemented with 0.05 *M* Tris–maleate buffer, and 0.02 *M* nicotinamide, using glass homogenizers with Teflon pestles. Final volumes for the corticosterone production and reductase assays were 0.5 and 0.25 ml per beaker, respectively, and each beaker contained 10 mg adrenal tissue. Incubation time was 60 min. Corticosterone production was measured by acid fluorescence (23), and total corticosteroid production by the blue tetrazolium method (24). Adrenal 5$\alpha$-reductase activity was measured using separate tissue aliquots after addition of 12.5 $\mu$g corticosterone to each vessel (22). Acid fluorescence (23) was used to determine the amount of steroid remaining after incubation. The statistical significance of the results was determined in Experiment I by use of Student's *t* test and in Experiment II by factorial analysis of variance (25).

*Results and Discussion.* Progesterone capsule weight loss was similar in both experiments (Tables I and II) and plasma progesterone levels were significantly increased in all the treated groups. Progesterone administration to intact rats decreased adrenal weight, plasma corticosterone concentration, and *in vitro* corticosterone and total corticosteroid production (Table I). These observations are consistent with decreased ACTH secretion. Holub, Katz, and Jailer (26) reported that the synthetic progesten, 6-methyl-17 acetoxyprogesterone, lowered pituitary ACTH output in response to acute stress; other authors have reported progestens cause adrenal atrophy (7, 8, 12). Progesterone may decrease ACTH release by effects on appropriate hypothalamic and/or pituitary receptor sites. Alternatively, its effect could be mediated by diminution of estrogen secretion (16), a possibility supported by the observed decrease in uterine weight (Table I). Coyne and Kitay (1) have shown that estrogen secretion in the rat is necessary for maintenance of normal ACTH levels. Moreover, estradiol has been shown to affect adrenal function directly (2) as well as through modification of ACTH secretion. Finally, progesterone per se could inhibit adrenal steroid production.

Administration of progesterone to ovariectomized rats decreased corticosterone and total corticosteroid production by adrenal homogenates, but did not significantly affect any other measurement of adrenal function (Table II). These data indicate that progesterone administered *in vivo* may affect *in vitro* adrenal function independently of an effect on estrogen secretion. As in the intact female, this influence may be exerted directly at the adrenal level or mediated through an effect on ACTH secretion.

No significant effect of progesterone was demonstrated with respect to adrenal 5$\alpha$-reductase activity in either intact (Table I) or ovariectomized (Table II) rats, indicating that progesterone does not share this site of action on adrenal function with the other gonadal hormones. A secondary inhibition of the activity of the enzyme might be expected in the intact rat since progesterone apparently decreases both estrogen and ACTH secretion as noted above; both these hormones suppress adrenal 5$\alpha$-reductase activity (2, 27). However, the observed changes in uterine and adrenal weight seen in the progesterone-treated intact rats were small. Thus, the dose of progesterone administered may have been insufficient to cause a detectable increment in adrenal reductase activity. Progesterone might act as a competitive substrate with corticosterone for the enzyme; however, no evidence of such competition was demonstrated. Progesterone treatment had no effect on the amount of corticosterone reduced in any treated group, even when significant levels of reductase activity were present, as in the ovariectomized–progesterone-treated animals.

Estrogen treatment of ovariectomized animals decreased body weight and adrenal 5$\alpha$-reductase activity, and increased uterine weight, adrenal weight, and *in vitro* corticosterone and total corticosteroid production

TABLE II. Effects of Progesterone and Estradiol in Ovariectomized Female Rats.[a]

| Measurement | Treatments | | | |
|---|---|---|---|---|
| | Control | Progesterone | Estrogen | Progesterone-Estrogen |
| Body wt (g) | 262 ± 5 | 252 ± 4 | 229 ± 3[e] | 234 ± 8 |
| Capsule wt loss (mg/10 day) | — | 19.4 ± 1.0 | — | 20.3 ± 0.7 |
| Plasma progesterone (μg/100 ml) | 0.5 ± 0.1 | 4.5 ± 0.4[d] | 0.5 ± 0.1 | 3.7 ± 0.3 |
| Adrenal wt (mg) | 69 ± 2 | 58 ± 2 | 75 ± 3[d] | 80 ± 5 |
| Plasma corticosterone (μg/100 ml) | 15.4 ± 3.2 | 7.0 ± 3.0 | 18.0 ± 2.8 | 14.4 ± 4.8 |
| Homogenate corticosterone production (μg/10 mg/hr) | 1.2 ± 0.1 | 0.6 ± 0.1[d] | 2.3 ± 0.2[e] | 1.7 ± 0.3 |
| Homogenate total corticosteroid production (μg/10 mg/hr) | 2.7 ± 0.2 | 2.2 ± 0.1[c] | 2.8 ± 0.2 | 2.5 ± 0.3 |
| Reductase activity[b] | 5.0 ± 0.9 | 6.0 ± 0.7 | 14.3 ± 0.5[e] | 13.4 ± 0.3 |
| Uterine wt (mg) | 71 ± 7 | 96 ± 10 | 278 ± 14[e] | 257 ± 22 |

[a] Results are shown as means and standard errors of the mean. All values are based on seven subjects per group.

[b] Expressed as micrograms corticosterone recovered per beaker, 12.5 μg having been added at the outset of incubation.

[c] $P < 0.05$; for the main effect of the treatment indicated, across groups receiving the treatment.

[d] $P < 0.01$; for the main effect of the treatment indicated, across groups receiving the treatment.

[e] $P < 0.001$; for the main effect of the treatment indicated, across groups receiving the treatment.

(Table II) as previously documented (1, 2). There was no significant interaction between the estrogen and progesterone treatments in the ovariectomized animals.

*Summary.* Progesterone treatment *in vivo* caused decreased corticosterone and corticosteroid production by homogenates from intact and ovariectomized female rats. Uterine weight, adrenal weight, and plasma corticosterone levels were also reduced by progesterone treatment of intact females. The effect of progesterone in ovariectomized rats indicates that the action of this hormone on *in vitro* adrenal function may be independent of any modulation of estrogen secretion. Progesterone, in contrast to estradiol and testosterone, has no significant effect on adrenal 5$\alpha$-reductase activity.

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