NOTE

No Inhibitory Effects of Gestrinone and Medroxyprogesterone Acetate on the Estrogen Production by Ovaries of Hypophysectomized Rats Stimulated by Gonadotropins

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Abstract. The in vivo effects of gestrinone (R2323) and medroxyprogesterone acetate (MPA) on the estrogen production by rat ovaries were investigated. Hypophysectomized immature female rats treated with 2.5 or 5 IU of pregnant mare serum gonadotropin (PMS) were daily given vehicle only, gestrinone (0.5 mg/kg body weight) or MPA (10 mg/kg body weight), and the activities of 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, 17, 20-lyase, 17β-hydroxysteroid dehydrogenase and aromatase in ovaries of these rats were measured. Gestrinone suppressed the 3β-hydroxysteroid dehydrogenase activity and increased activities of 17α-hydroxylase, 17, 20-lyase and aromatase in ovaries stimulated by 5 IU of PMS, while MPA suppressed activities of 17α-hydroxylase and aromatase in these ovaries. On the other hand, the aromatase activity in ovaries stimulated by 2.5 IU of PMS was suppressed by gestrinone and increased by MPA, and neither gestrinone nor MPA affected the production of aromatizable androgens from progesterone by these ovaries. Thus, gestrinone and MPA administrated in vivo showed divergent influences on steroidogenic enzyme activities in ovaries, but they did not affect the serum concentration of estradiol-17β. The present results suggest that neither gestrinone nor MPA reduced estrogen production by the rat ovary under the gonadotropin stimulation although they influenced some process of its steroidogenesis.

Key words: Gestrinone, Medroxyprogesterone acetate, Ovary, Estrogen, Rat.

ENDOMETRIOSIS is a gynecological disorder that has been defined as the presence of a functioning endometrial gland and stroma outside the uterine cavity, and its development depends on the ovarian hormones. Based on the hormonal requirements of endometriotic tissue, various pharmacological agents have been developed for the medical management of endometriosis, and the possible mechanism by which these agents are effective is assumed to reduce the serum levels of estrogen or to antagonize the action of ovarian hormones in endometriotic tissue.

Gestrinone (R2323; 17α-13-ethyl-17-hydroxy-18, 19-dinor-pregna-4, 9, 11-trien-20-yn-3-one) which has estrogenic, androgenic, progestomimetic, anti-estrogenic and anti-progestational activities [1, 2], is effective in the treatment of endometriosis [3-5]. Robyn et al. [6] reported that gestrinone at a dose of 2.5 mg twice a week slightly decreased the serum level of estradiol-17β without any changes in the serum levels of LH or FSH,
suggesting the direct inhibitory effect of gestri-
none on estrogen production in the ovary. How-
ever, in contrast to the results of Robyn et al. [6],
several studies showed that gestrinone at a dose of
2.5 mg twice a week did not reduce the serum level
of estradiol-17β [2, 7]. Thus, the direct inhibitory
action of gestrinone on estrogen production in the
ovary in vivo is controversial.

Medroxyprogesterone acetate (MPA; 6α-
methyl-17α-hydroxyprogesterone acetate), a po-
tent progestational steroid, is also effective in the
treatment of endometriosis [8], and its direct
inhibitory action on estrogen production in the
ovary in vivo is unknown. To examine the direct
effects of gestrinone and MPA on the ovary in vivo,
we investigated their effects on estrogen produc-
tion by ovaries of hypophysectomized immature
female rats stimulated by pregnant mare serum
gonadotropin.

Materials and Methods

Chemicals

Chemicals were obtained from the following
sources; [1, 2, 6, 7-3H] progesterone (82 Ci/mmol),
[1, 2, 6, 7-3H]androstenedione (115 Ci/mmol), [1,
2, 6, 7-3H]testosterone (88 Ci/mmol), [1, 2-3H]17α-
hydroxyprogesterone (57.4 Ci/mmol) and [4, 7-
3H] pregnenolone (20.5 Ci/mmol) from Amer-
sham International Plc. (Buckinghamshire, Eng-
land), [1-3H] androstenedione (27.8 Ci/mmol)
from New England Nuclear Co. (Boston, MA,
U.S.A.), non-radioactive steroids from Steraloids
Inc. (Pawling, NY, U.S.A.) and digitonin from
Sigma Chemical Co. (St. Louis, MO, U.S.A.).
Pregnant mare serum gonadotropin (PMS), gestri-
one (R2323) and medroxyprogesterone acetate
(MPA) were kindly provided by Teikoku Hor-
mone Co. (Minato, Tokyo, Japan), Roussel-Uclaf
(Paris, Romainville, France), and Kyowa Hakko
Co., Ltd. (Minato, Tokyo, Japan), respectively.

Animals

Female Wistar/ST rats purchased from Japan
SLC (Hamamatsu, Shizuoka, Japan) were
hypophysectomized at the age of 28 days by the
external auditory canal method under sodium
pentobarbital anaesthesia as described previously
[9]. These rats were divided into 3 groups and
daily injected subcutaneously with 2.5 or 5 IU of
PMS dissolved in 0.1 ml of saline for 14 days from
the age of 35 days. Each group of rats were
simultaneously given vehicle only (0.5% (w/v) CM
cellulose in saline), gestrinone (0.5 mg/kg body
weight) or MPA (10 mg/kg body weight) in a
volume of 0.1 ml by gastric gavage for the same
period. All rats at the age of 49 days were killed 24
h after the last treatment, and complete removal of
the hypophyses was confirmed. Serum was
obtained after the centrifugation of blood drawn
from the descending aorta at × 1000 g for 10 min.
The ovaries and uterus were also removed. The
serum and ovaries were stored at −80°C until
used. Some ovaries and uteri were fixed in 10%
phosphate buffered formalin (pH 7.2) for histology.
All rats were kept at 25°C under light control
(12 h light/12 h darkness) and allowed free access
to water and pellet food.

3β-Hydroxysteroid dehydrogenase activity

The activity of 3β-hydroxysteroid dehydro-
genase was measured according to the method
of Berko et al. [10]. Ovaries were homogenized in
0.01 M potassium phosphate buffer (pH 7.4)
containing 0.25 M sucrose, 20 mM EDTA and
20% glycerol. 0.05 ml of [3H]pregnenolone (4
μCi/50 nmol) dissolved in ethanol was then added
to each reaction tube containing 0.9 ml of the
ovarian homogenate (5 mg tissue) and 0.05 ml of 5
mM NAD+, and the mixed solution was incubated
at 37°C for 4 min. After the incubation, 0.5 ml of
the reaction solution was transferred into a tube
containing 0.5 ml of carrier pregnenolone (2
mg/ml in ethanol), and the amount of
[3H]progesterone in the solution was determined
by precipitating pregnenolone with digitonin.

Activities of 17α-hydroxylase, 17, 20-lyase and 17β-
hydroxysteroid dehydrogenase

The procedures for the incubation and the
extraction of steroids were the same as those
described previously [11]. Ovaries were homoge-
nized in 0.25 M sucrose (pH 7.4) containing 1 mM
EDTA. 0.2 ml of ovarian homogenate was then
added to each incubation tube with 0.8 ml of 0.3 M
potassium phosphate buffer (pH 7.4) containing
1.3 mM NADPH, 0.06 M nicotinamide, 2 mM
MgCl₂ and the ³H-labeled substrate, and the reaction solution was incubated at 37°C for 4 min. [³H]Progesterone (1 µCi/ nmol) and 1.5 mg ovarian tissue, [³H]17α-hydroxyprogesterone (1 µCi/ nmol) and 20 mg ovarian tissue, and [³H]androstenedione (1 µCi/ nmol) and 20 mg ovarian tissue were used for assays of activities of 17α-hydroxylase, 17, 20-lyase and 17β-hydroxysteroid dehydrogenase, respectively. After the extraction of [³H]steroids from the incubation solution, the extracted steroids were subjected to silica gel thin layer chromatography in a solvent system of benzene: acetone (4:1, v/v) and separated into progesterone, androstenedione, 17α-hydroxyprogesterone and testosterone fractions. [³H]Steroids in the 17α-hydroxyprogesterone fraction were further subjected to thin layer chromatography in a solvent system of hexane: ethylacetate (1:1, v/v). [³H]Steroids extracted from each fraction were finally recrystallized to constant specific activity with a non-radioactive steroid corresponding to each fraction. The fraction of [³H]testosterone was recrystallized after acetylation. The recovery rate for each [³H]steroid was estimated by seeking the recovery rate for [³H]androstenedione (0.2 µCi/0.2 nmol), [³H]17α-hydroxyprogesterone (0.2 µCi/0.2 nmol) or [³H]testosterone (0.2 µCi/0.2 nmol) mixed with 0.2 ml of ovarian homogenate and 0.8 ml of the potassium phosphate buffer. The amount of each [³H]steroid produced from 3H-labeled substrate was corrected by each recovery rate. Activities of 17α-hydroxylase, 17, 20-lyase and 17β-hydroxysteroid dehydrogenase were expressed as the amount of androstenedione plus 17α-hydroxyprogesterone/mg tissue/min, androstenedione/mg tissue/min, and testosterone/mg tissue/min, respectively.

Aromatase activity

Aromatase activity was assayed according to the method described by Tsuji et al. [12]. Ovaries were homogenized in 0.15 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 10 mM dithiothreitol. 0.5 ml of the ovarian homogenate (5 or 20 mg tissue) was then added to each reaction tube with 0.5 ml of the homogenizing buffer containing [¹³C]androstenedione (1 µCi/0.5 nmol) and 1.3 mM NADPH, and the solution was incubated at 37°C for 1 or 2 h. After the incubation, the amount of [³H]H₂O released from [³H]androstenedione was measured.

Conversion of progesterone into aromatizable androgens

The procedures for the incubation, the extraction of steroids, the separation of steroids and the recrystallization of each separated steroid were the same as those described for the measurement of each enzyme activity except that 20 mg ovarian tissue and [³H]progesterone (1 µCi/ nmol) were used for the incubation and that the incubation time was 1 h.

Assay of serum estradiol-17β

The concentration of serum estradiol-17β was determined with a radioimmunoassay kit (Diagnostic Products Co., Los Angeles, CA, U.S.A.). The intra- and inter-assay coefficients of variation were 6.0 and 6.8%, respectively.

Statistics

Values are presented as the means ± SE, and Student’s t-test was used for the statistical analysis.

Results

Effects of gestrinone and MPA on the weights of the body, both ovaries and uterus

Hypophysectomized immature rats treated daily with 2.5 or 5 IU of PMS were daily given vehicle only, gestrinone or MPA. There was no significant difference between the body weight of rats treated with 2.5 and 5 IU of PMS, and gestrinone and MPA had no influence on body weight (Table 1). The treatment with 5 IU of PMS increased the ovarian weight markedly, and the ovarian weight of rats treated with 5 IU of PMS was much higher than that of mature female rats at 10 weeks old (59.5±8.5 mg; mean ± SE, n=9), but the ovarian weight of rats treated with 2.5 IU of PMS was slightly lighter. The uterine weight of rats treated with 5 IU of PMS was slightly heavier than that of rats treated with 2.5 IU of PMS, but the difference was not significant. Gestrinone had no effect on the uterine weight of rats. On the other hand, MPA slightly decreased the uterine weight of rats.
Table 1. Effects of gestrinone and MPA on the weights of body, both ovaries and uterus

<table>
<thead>
<tr>
<th>PMS (IU)</th>
<th>Agent</th>
<th>No. of rat</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Body (g)</td>
</tr>
<tr>
<td>2.5</td>
<td>Vehicle</td>
<td>7</td>
<td>86.2±3.1</td>
</tr>
<tr>
<td></td>
<td>Gestrinone</td>
<td>7</td>
<td>82.2±2.6</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>7</td>
<td>83.2±2.7</td>
</tr>
<tr>
<td>5.0</td>
<td>Vehicle</td>
<td>7</td>
<td>85.5±4.5</td>
</tr>
<tr>
<td></td>
<td>Gestrinone</td>
<td>4</td>
<td>85.9±7.9</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>8</td>
<td>87.2±3.1</td>
</tr>
</tbody>
</table>

Three groups of hypophysectomized female rats at the age of 35 days were injected daily with 2.5 or 5 IU of PMS for 14 days. Each group of rats was given daily gestrinone, MPA or vehicle only during the same period. All rats were killed at the age of 49 days.

*a, Mean±SE; b, P<0.05, significant difference from the values for rats treated with 2.5 IU of PMS.

treated with 2.5 IU of PMS, while it had no effect on the uterine weight of rats treated with 5 IU of PMS. Neither gestrinone nor MPA influenced the histology of ovaries (data not shown).

Effects of gestrinone and MPA on activities of steriodogenic enzymes

Activities of enzymes involved in the production of estrogen from pregnenolone were compared in rats given vehicle only, and gestrinone or MPA under the treatment with 5 IU of PMS (Table 2). Gestrinone suppressed the 3β-hydroxysteroid dehydrogenase activity to about a half of that in the ovaries of rats given vehicle only, whereas it increased the activities of 17α-hydroxylase, 17, 20-lyase and aromatase. 17β-Hydroxysteroid dehydrogenase activity was not affected by gestrinone. MPA decreased the activities of 17α-hydroxylase and aromatase significantly, but did not affect the activities of 3β-hydroxysteroid dehydrogenase, 17,20-lyase or 17β-hydroxysteroid dehydrogenase.

Neither gestrinone nor MPA influenced the 3β-hydroxysteroid dehydrogenase activity in ovaries of rats treated with 2.5 IU of PMS whereas gestrinone decreased the aromatase activity in these ovaries and MPA increased it (Table 3). The production of ovarian steroids stimulated by 2.5 IU of PMS was limited so that the production of

Table 2. Effects of gestrinone and MPA on activities of steroidogenic enzymes in ovaries stimulated by 5 IU of PMS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (p mol/mg tissue/min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>3β-Hydroxysteroid dehydrogenase</td>
<td>5.20±0.05×10²</td>
</tr>
<tr>
<td>17α-Hydroxylase</td>
<td>1.47±0.07×10</td>
</tr>
<tr>
<td>17, 20-Lyase</td>
<td>1.61±0.06×10⁻¹</td>
</tr>
<tr>
<td>17β-Hydroxysteroid dehydrogenase</td>
<td>2.35±0.17×10⁻¹</td>
</tr>
<tr>
<td>Aromatase</td>
<td>3.00±0.02×10⁻²</td>
</tr>
</tbody>
</table>

Three groups of hypophysectomized female rats at the age of 35 days were daily injected with 5 IU of PMS for 14 days. Each group of rats was given daily gestrinone, MPA or vehicle only for the same period. All rats were killed at the age of 49 days, and the ovarian enzyme activities were measured. *Mean±SE of 4 determinations; b, P<0.05, significant difference from the values for rats given vehicle only.
Table 3. Effects of gestrinone and MPA on activities of 3β-hydroxysteroid dehydrogenase and aromatase in ovaries stimulated by 2.5 IU of PMS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Vehicle</th>
<th>Gestrinone</th>
<th>MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-Hydroxysteroid dehydrogenase</td>
<td>2.78±0.16×10³</td>
<td>3.13±0.22×10³</td>
<td>2.60±0.13×10³</td>
</tr>
<tr>
<td>Aromatase</td>
<td>2.40±0.07×10³</td>
<td>0.83±0.04×10⁻³</td>
<td>3.56±0.16×10⁻³</td>
</tr>
</tbody>
</table>

Three groups of hypophysectomized female rats at the age of 35 days were injected daily with 2.5 IU of PMS for 14 days. Each group of rats was given daily gestrinone, MPA or vehicle only for the same period. The rats were killed at the age of 49 days, and the ovarian enzyme activities were measured. a, Mean±SE of 4 determinations; b, P<0.05, significant difference from the values for rats given vehicle only.

Table 4. Effects of gestrinone and MPA on the conversion of progesterone into aromatizable androgens in ovaries stimulated by 2.5 IU of PMS

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Vehicle</th>
<th>Gestrinone</th>
<th>MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>58.9±8.5</td>
<td>68.2±6.9</td>
<td>55.6±10.5</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5.1±1.8</td>
<td>7.5±1.6</td>
<td>13.6±2.9</td>
</tr>
<tr>
<td>Androstenedione plus testosterone</td>
<td>64.0±10.3</td>
<td>75.7±8.5</td>
<td>69.2±13.4</td>
</tr>
</tbody>
</table>

Three groups of hypophysectomized female rats at the age of 35 days were injected daily with 2.5 IU of PMS for 14 days. Each group of rats was given daily gestrinone, MPA or vehicle only for the same period. The rats were killed at the age of 49 days. The conversion of progesterone into aromatizable androgens was estimated by incubating the ovarian homogenate (20 mg tissue equivalent) with [3H] progesterone (1 μCi/nmol) for 1 h in the presence of NADPH. a, Mean±SE of 3 determinations.

Table 5. Effects of gestrinone and MPA on the concentration of serum estradiol-17β

<table>
<thead>
<tr>
<th>PMS (IU)</th>
<th>Agent</th>
<th>No. of rats</th>
<th>Concentration of estradiol-17β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>Vehicle</td>
<td>7</td>
<td>60.9±9.8</td>
</tr>
<tr>
<td></td>
<td>Gestrinone</td>
<td>7</td>
<td>58.0±8.8</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>7</td>
<td>70.2±6.2</td>
</tr>
<tr>
<td>5.0</td>
<td>Vehicle</td>
<td>10</td>
<td>714.3±171.0p</td>
</tr>
<tr>
<td></td>
<td>Gestrinone</td>
<td>8</td>
<td>761.9±158.6p</td>
</tr>
<tr>
<td></td>
<td>MPA</td>
<td>9</td>
<td>674.0±113.6p</td>
</tr>
</tbody>
</table>

Three groups of hypophysectomized female rats at the age of 35 days were injected daily with 2.5 or 5 IU of PMS for 14 days. Each group of rats was given daily gestrinone, MPA or vehicle only for the same period. The rats were killed at the age of 49 days. a, Mean±SE; b, P<0.01, significant difference from the values for rats treated with 2.5 IU of PMS. androstenedione and testosterone from progesterone was examined to estimate the overall effects of gestrinone and MPA on the activities of 17α-hydroxylase, 17,20-lyase and 17β-hydroxysteroid dehydrogenase instead of measuring the activity of each enzyme (Table 4). Gestrinone and MPA had no significant effects on the production of androstenedione or testosterone (aromatizable androgens) from progesterone.

Effects of gestrinone and MPA on the concentration of serum estradiol-17β (Table 5)

The concentration of serum estradiol-17β in rats treated with 5 IU of PMS was much greater than that in rats treated with 2.5 IU of PMS. Gestrinone and MPA had no significant effects on the concentration of serum estradiol-17β in rats treated with
either 2.5 or 5 IU of PMS.

**Discussion**

Gestrinone and MPA influenced the activities of some ovarian enzymes of rats stimulated by 2.5 or 5 IU of PMS. Furthermore, the average ovarian weight of rats stimulated by 5 IU of PMS was slightly reduced by gestrinone and MPA, although not significantly. These results indicate that doses of gestrinone and MPA adopted in the study are enough to have some effects on the activities of ovarian enzymes. Thus, failure to detect their effects on the level of serum estradiol-17β is not due to inadequate doses of gestrinone and MPA but due to their weak actions on an overall process in estrogen production in the ovary.

The ovarian weight of rats treated with 5 IU of PMS was much greater than that of mature female rats, and the ovarian weight of rats treated with 2.5 IU of PMS was slightly less. Thus, treatments with 5 and 2.5 IU of PMS resulted in the hypertrophy and hypotrophy of ovaries, respectively. However, in either case, gestrinone and MPA showed no significant effects on the serum levels of estradiol-17β. These results indicate that gestrinone and MPA had little effect in vivo on estrogen production in the ovary either under excess or deficient stimulation by gonadotropins.

Kharbanda et al. reported that gestrinone at concentrations of 0.01–10 μM did not reduce the production of estradiol-17β from endogenous substrates by cultured goat granulosa cells and corpus luteum cells [13], although Arakawa et al. showed that gestrinone inhibited activities of the cholesterol side chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase and 17, 20-lyase prepared from rat ovaries [14, 15]. The report of Kharbanda et al. is in agreement with the present results showing that gestrinone administrated in vivo did not reduce the serum level of estradiol-17β.

Raynaud et al. showed that MPA and gestrinone reduced the mouse uterine weight increased by estrogen and that this anti-estrogenic activity of MPA was about 10 times greater than that of gestrinone [16]. Thus, a slight decrease due to MPA in the uterine weight of rats stimulated by 2.5 IU of PMS seems to be due to its anti-estrogenic activity. On the other hand, the inability of MPA to reduce the average uterine weight of rats stimulated by 5 IU of PMS may be ascribed to the marked increase in the serum level of estradiol-17β.

Oral doses of gestrinone and MPA for the treatment of endometriosis are 2.5 mg twice weekly [2, 4, 17] and 30 mg/day [18, 19], respectively. In the present study, gestrinone and MPA were administrated at doses about 10 times as great as these doses, but they did not reduce the serum levels of estradiol-17β. Although the difference between the absorption, metabolism and clearance of these drugs in human beings and rats should be taken into consideration, the present results suggest that these drugs at doses used for therapy may not reduce estrogen production by the ovary through their direct actions on the ovary.

**Acknowledgements**

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**References**

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EFFECTS OF GESTRINONE AND MPA


