EFFECTS OF NORETHYNNODREL AND MESTRANOL ON PITUITARY LUTEINIZING HORMONE IN THE FEMALE RAT

ANANT P. LABHSETWAR

Department of Anatomy, Washington University School of Medicine, St Louis, Missouri, U.S.A.

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Summary. The effects on the pituitary LH levels of norethynodrel (20 µg/rat or approximately 0.1 mg/kg daily) and mestranol (1 µg/rat or approximately 5 µg/kg daily) administered subcutaneously singly or in combination for 10 days to intact female rats have been studied. LH was assayed by the ovarian ascorbic acid depletion method of Parlow (1958). Norethynodrel caused a significant increase in hypophysial LH stores without causing ovarian atrophy or reducing compensatory hypertrophy in unilaterally ovariec- tomized post-pubertal rats. Mestranol administration, on the other hand, resulted in a significant ovarian atrophy without significant changes in the pituitary LH level, and also resulted in an inhibition of compensatory hypertrophy. The combined treatment resulted in effects essentially similar to those observed in the mestranol-treated group. No evidence for potentiation between the two steroids was obtained.

Data support the conclusion that when the two steroids are administered together in the dosages used, the oestrogenic component is primarily involved in the pituitary LH inhibition.

INTRODUCTION

The exact mechanism by which synthetic progestagens exert antifertility effects is not clear. One of their major actions is considered to be inhibition of luteinizing hormone (LH) release from the pituitary gland (Pincus, 1965). However, there is a paucity of data on the effects of these steroids on pituitary LH level. Labhsetwar (1966a) found a significant decrease in pituitary LH stores of intact female rats treated with medroxyprogesterone acetate. Others have used ovariec- tomized rats and/or assays which do not discriminate between follicle stimulating hormone (FSH) and LH (Saunders, 1964; Husain & Pincus, 1965). It is necessary to use specific assays since non-specific assays, although informative, sometimes lead to erroneous conclusions due to interaction between LH and FSH (Parlow, 1964). In the present study norethynodrel (17α-ethynyl-17α-hydroxy-Δ4(10)-estradiol-3-one) and mestranol (17α-ethynyl-3-methoxy-Δ1,3,5(10)-estratriene-17β-ol), progestagenic and oestrogenic components,
respectively, of the commercially marketed contraceptive preparation, Enovid R, have been used. They were administered to rats in dosages considered on a weight basis to fall in a range employed for fertility control in the human. The effect of these steroids on the output of gonadotrophins from the pituitary gland when administered singly or in combination was also studied. Preliminary data have appeared elsewhere (Labhsetwar, 1966b).

MATERIALS AND METHODS
Adult female rats of the Holtzman strain, weighing between 200 and 225 g, were caged in a room maintained at a constant temperature with 14 hr of artificial light daily and supplied generously with Purina laboratory chow and tap-water. They were divided into four groups. One group received norethynodrel (20 µg/rat or approximately 0·1 mg/kg daily), the second group received mestranol (1 µg/rat or approximately 5 µg/kg daily) and a third group received a combination of the two, injected at two different sites. The fourth group served as controls. All injections were made in sesame oil subcutaneously once a day for 10 days.

At autopsy, ovarian, uterine, adrenal, anterior pituitary and total body weights were recorded. The pituitary glands from two rats within each group were pooled to obtain four pools per group and stored in a freezer until the time of assay.

LH assay
The ovarian ascorbic acid depletion method of Parlow (1958) was used. The details of the assay have been described previously (Labhsetwar, 1966a). Typically, three assay rats per dose were employed. The assays were replicated four times. Each replicate contained one pituitary pool (2 mg/rat) from each experimental group and two doses (4 and 20 µg) of reference standard (NIH-LH-s-5, ovine).

Gonadotrophin output
This output was evaluated by measuring the ovarian compensatory hypertrophy. In the first experiment, 35-day-old Holtzman female rats (80 to 100 g) were unilaterally ovariectomized by removing the left ovary. The same steroids were injected for 9 days. On a weight basis the dosages were of the same order as in the adult rats. The experiment was repeated later using 37-day-old rats (90 to 100 g) of the same strain. At autopsy the number of corpora lutea and the ovarian weights were recorded. In addition, in the second experiment, the ovaries, uteri, adrenals and anterior pituitary glands were removed, trimmed and weighed. The ovarian compensatory hypertrophy was calculated by comparing the weight of the second ovary with that of the first (left) removed from the same animal. It is presumed that these animals were under the influence of exogenous steroids at the time of LH release coincident with post-pubertal ovulations, for vaginal opening occurs in a majority of these animals when they are about 40 days old. Inhibitory effects of the administered steroid, if any, would therefore be reflected in a decreased luteal count.
### Table 1
EFFECTS OF NORETHYNODREL AND MESTRANOL ON ORGAN WEIGHTS AND PITUITARY LEVELS IN ADULT AND 37-DAY-OLD RATS

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>37-day-old†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Norethynodrel</td>
</tr>
<tr>
<td>No. of rats</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ovarian weight (mg)</td>
<td>79 ± 4.0†</td>
<td>72 ± 3.9</td>
</tr>
<tr>
<td>Pituitary weight (mg)</td>
<td>8.9 ± 0.03</td>
<td>8.5 ± 0.51</td>
</tr>
<tr>
<td>LH concentration§ (μg/mg wet pituitary)</td>
<td>1.82 ± 1.05</td>
<td>3.72 ± 1.10*</td>
</tr>
<tr>
<td>LH content (μg/gland)</td>
<td>17.3</td>
<td>35.8</td>
</tr>
</tbody>
</table>

* P<0.05 when compared with respective control.
† Unilaterally ovariectomized when 37 days old.
‡ Mean ± S.E. throughout Table.
§ μg equivalents of NIH-LH-s-5 (ovine); four pools of pituitary glands were assayed for each group.
Analysis of data

Analysis of variance was used and significant differences between means were detected by application of Duncan’s multiple range test (Steel & Torrie, 1960). A further analysis by casting the data into a factorial design yielded exactly the same results.

RESULTS

Organ weights

Norethynodrel did not significantly affect the ovarian or pituitary weights (Table 1). Mestranol administration, on the other hand, resulted in a significant decrease in the ovarian weight and an increase in the pituitary weight when compared with the control. The combined treatment did not result in a significant decline in the ovarian weight when compared with the mestranol-treated group. In the unilaterally ovariectomized postpubertal rats, data on organ weights essentially substantiate those obtained in the adult animals. The uterine, adrenal and body weights in adult as well as in post-pubertal rats were not significantly affected by any of the treatments.

| Table 2 |
|-----------------|-----------------|-----------------|-----------------|
|                  | Control         | Norethynodrel   | Mestranol       | Norethynodrel+mestranol |
| No. of rats      | 10              | 9               | 12              | 11                |
| No. with c.L.    | 9               | 8               | 10              | 7                 |
| No. of c.L./rat  | 9·9±1·3†        | 11·2±0·6        | 5·2±1·3*        | 4·4±1·2*          |
| % increase in ovarian weight | 251±29         | 225±21         | 133±15         | 91±16             |

* P<0·05 when compared with the control.
† Mean ± S.E. throughout Table.

LH level

Norethynodrel treatment resulted in a significant increase in the hypophysial LH concentration (μg/mg) when compared with the control (Table 1). When norethynodrel was combined with mestranol, a significant decline in LH concentration resulted, although mestranol by itself exerted no significant effect (Table 1). The difference between the mestranol-treated group and the group receiving a combination of steroids was not significant.

The total LH content of the gland followed essentially the same pattern as that of LH concentration (Table 1).

Gonadotrophin output

Results obtained in the post-pubertal rats of both experiments were essentially similar and have been pooled (Table 2). None of the ovaries removed at ovariection contained visible corpora lutea, and all animals had closed vaginas. Ten days later, all the controls as well as treated animals had open
vaginae, and their ovaries contained variable numbers of corpora lutea (Table 2).

Mestranol, but not norethynodrel, caused a significant inhibition of ovarian hypertrophy together with a decrease in the number of corpora lutea (Table 2). The combined treatment did not result in a significantly more severe inhibition of ovarian hypertrophy than that caused by mestranol alone. The number of corpora lutea in the residual ovary paralleled the inhibition of compensatory hypertrophy.

DISCUSSION

The daily dose of norethynodrel (about 0.1 mg/kg) used in this study has been previously shown to be inadequate to block ovulation, fertility and nidation in the adult rat upon subcutaneous administration (Saunders, 1964; Desaulles & Krähenbuhl, 1964). The uterotrophic effect of this dose of norethynodrel was also negligible (Table 1) and it was found ineffective in altering the ovarian sensitivity to gonadotrophins (Saunders & Drill, 1958; Eckstein & Mandl, 1962). Thus, although this dose has been found incapable of exerting a significant peripheral action, the present study suggests that it is effective in increasing hypophysial LH stores. The mechanism by which norethynodrel enhances pituitary LH stores is speculative. The effect appears to be reminiscent of increase in the pituitary LH level in response to administration of natural progestagen—progesterone (Hoffman & Schwartz, 1965; Van Rees & de Groot, 1965). This increase could result from an inhibition of LH release, a stimulation of LH synthesis, or both. The failure of this dose of norethynodrel to block formation of corpora lutea in the adult (Saunders, 1964), immature (Shipley & Meyer, 1965) or post-pubertal rat (Table 2) suggests that it is inadequate to reduce LH release from the pituitary gland. It, therefore, appears that norethynodrel, when administered in smaller doses for a relatively short duration, may stimulate LH synthesis in the pituitary gland. This could be due to its direct action on the pituitary gland or hypothalamus or both.

Norethynodrel may possibly increase pituitary LH stores by its ability to induce pseudopregnancy. Pseudopregnancy in the rat is known to result in increased pituitary LH stores (Schwartz & Rothchild, 1964; Van Rees & de Groot, 1965) and norethynodrel has been found capable of inducing pseudopregnancy in the cyclic rat (Labhsetwar, 1966c). The LH level in the norethynodrel-treated group (3.7 µg/mg) is comparable to that found on Days 7 to 8 of pseudopregnancy (3.3 µg/mg; Labhsetwar, 1967).

In contrast with the effects of norethynodrel, mestranol exerted no significant effects on hypophysial LH stores, but caused a significant ovarian atrophy (Table 1) and reduction in both the number of corpora lutea and the degree of ovarian hypertrophy in the post-pubertal rats (Table 2). This suggests that LH output from the pituitary gland was reduced. It is obvious that mestranol blocked the synthesis of LH in the pituitary gland, since the reduced output did not result in its accumulation in the gland.

The effect of norethynodrel in increasing the pituitary LH store disappeared when it was combined with mestranol. In fact, LH stores in the group receiving
both steroids were significantly lower than those in the group receiving norethynodrel alone. The LH level, ovarian weight (Table 1), compensatory hypertrophy and number of corpora lutea (Table 2) in the group receiving the two steroids in combination were not significantly different from those in the mestranol-treated group. Thus it appears that, when the two steroids are administered together in the dosages used, the oestrogenic component acts as a primary agent responsible for pituitary inhibition. Orally administered norethynodrel, as used for contraceptive purposes, is known to undergo conversion to oestrogenic derivatives (Paulsen, Leach, Lanman, Goldston, Maddock & Heller, 1962; Bialy, Layne & Pincus, 1965). This conversion may further reinforce the pituitary inhibition caused by mestranol. A significant ovarian atrophy (Table 1) and an inhibition of compensatory hypertrophy (Table 2) in both mestranol and mestranol–norethynodrel-treated groups, but not in the norethynodrel-treated group, suggests that mestranol may have decreased FSH output in addition to that of LH.

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REFERENCES
Norethynodrel, mestranol and pituitary LH in the rat


