Influences of Ethynodiol Diacetate (SC 11800) on Estrous Cycle and Pituitary Gonadotropin

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Ethynodiol diacetate (SC 11800) has been used for progestin supplementing therapy for obstetric and gynecologic diseases because of its strong progestin action. Moreover, it has been used as an oral contraceptive because it has an ovulation inhibiting effect, on which many excellent clinical results have been reported. The present paper deals with some consideration on its mode of action on the basis of its influence on the estrous cycle and contents of pituitary gonadotropin in the female rat.

MATERIALS AND METHODS

One hundred and seventy-six mature female Wistar strain rats, weighing more than 150 g and having regular estrous cycle, were used. During 2 weeks before the experiment, the vaginal smear was also collected at 9 o’clock in the morning and the estrous cycle during the treatment was observed.

SC 11800, progesterone, estradiol benzoate and testosterone propionate were each dissolved in sesame oil. Each of the steroids was injected subcutaneously in a dose of 0.1 ml at the back of female rats every day for successive days. On the 22nd day the rats were sacrificed by decapitation and the pituitary was removed immediately. After weighing the anterior lobe, it was homogenized in 0.4 ml of physiological saline per one specimen.

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by grinding in an agate mortar and the homogenate was used for the determination of prolactin. For determinations of LH and FSH, the specimens were immersed in cooled acetone solution which was exchanged once 24 hours later, dehydrated on a filter paper 1 or 2 days later, and then dried up in a dessicator. Just before the determination, the specimens were homogenized in an agate mortar with physiological saline at final concentration of 3 mg/ml.

FSH was determined by the Steelman-Pohley (1953) method modified by Parlow (1964). Young female Wistar strain rats, aged 23 days and weighed 30 to 40 g, were used. 50 μg and 150 μg, or 50 μg and 100 μg of FSH standard substance (NIH-FSH-S4) were mixed with 50 I.U. of HCG (gonadotropin, Teikoku Zoki Pharmaceutical Co.). The hypophyseal homogenate was also mixed with 50 I.U. of HCG in the same way and was diluted with physiological saline so as to obtain the volume of 0.5 ml for one shot. Then, the mixture was injected into the femoral muscle of either side alternately twice daily, at 9 a.m. and 5 p.m., every consecutive 3 days. Seventy-two hours after the first injection, both ovaries were isolated, and the circumambient tissues were removed. The specimens were dehydrated by pressing them slightly on a filter paper, and weighed immediately with a torsion balance.

LH was determined by the modified ovarian ascorbic acid (OAA) depletion method of Parlow (1958). To young female rats, aged 24 to 26 days, 100 I.U. of PMS (Serotropin, Teikoku Zoki Pharmaceutical Co.) were injected as a pretreatment, and 50 I.U. of HCG were injected 64 hours later. The left ovary was isolated on the 7th day of the first HCG injection, and weighed with a torsion balance, and homogenized immediately with a glass homogenizer with 10 ml of 2.5% metaphosphate solution, and filtered with the Toyo filter paper No. 1. OAA in the filtrate was determined photometrically at 515 μm. The amount of ascorbic acid was estimated in terms of that contained in 100 g of the ovary, and LH was expressed as a decrease in OAA content in the right ovary estimated at the end of 2 hours after the administration of samples, using the left ovary as control.

Prolactin was determined according to Nicoll (1957). 0.1 ml of samples was subcutaneously injected on just above the left crop of pigeons, and physiological saline on the right as a control. They received 4 injections in total at 9 a.m. and 4 p.m. on the first and second day, and were sacrificed by decapitation at 3 p.m. on the third day. The crops were isolated immediately and sucking apparatus was spread on to scrape the mucous epithelium in the reacting region, and the mucous epithelium thus obtained was dried in an dessicator at 110°C for 24 hours and weighed. Prolactin was estimated as a difference in the weight between experimental group and control after drying.

RESULTS

1. Contents of pituitary gonadotropin during normal estrous cycles

The contents of pituitary gonadotropin during normal estrous cycles in the female rats were assayed. FSH and LH periodically changed mostly at the proestrus and prolactin mostly at the metaestrus. FSH ranged from 17.3 to 21.4 μg/mg, LH from 0.9 to 3.4 μg/mg, and prolactin from 21.3 to 81.5 mU/mg.

2. Estrous cycles in cases of SC 11800 administration

Several doses of SC 11800 were administered for every successive 21 days. In cases of 0.01 mg/day, the diestrus appeared rather frequently but without any marked changes. But, in cases of 0.1 mg/day, the estrus was likely to be inhibited, showing continuous diestrus. In cases of 0.5 mg/day and 1.0 mg/day, the longer continuous diestrus appeared.
Progesterone caused almost no changes in estrous cycle when it was administered at a dose of 0.02 mg/day, disturbed estrous cycles at a dose of 0.2 mg/day, and showed continuous complete diestrus at a dose of 2 mg/day.

Estradiol benzoate caused almost no changes in estrous cycle in cases of 0.1 µg/day and 10 µg/day, but showed continuous diestrus in cases of more than 100 µg/day.

When testosterone propionate was administered, the estrous cycle was not appreciably disturbed at first, but continuous diestrus was seen in proportion to the duration of treatment. But, when it was increased to 0.1 mg/day and 1 mg/day, the estrus was inhibited soon after the treatment, showing continuous diestrus.

3. Gonadotropin in cases of steroids administration

Table 1 shows contents of pituitary gonadotropin when several steroids were administered for 21 days. When estradiol benzoate was administered, FSH was markedly increased but LH was rather decreased. When progesterone and testosterone were administered, FSH was increased and LH showed its maximum level at a dose of 0.1 mg/day. The prolactin was also rather increased in proportion to the amount of administration.

**Table 1. Contents of pituitary gonadotropin when several steroids were administered for 21 days**

<table>
<thead>
<tr>
<th>Gonadotropin contents in the pituitary</th>
<th>FSH</th>
<th>LH</th>
<th>Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovarian wt. (mg)</td>
<td>µg/pit.</td>
<td>OAAD (%)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>1 µg</td>
<td>42.2±3.9</td>
<td>73.3±9.1</td>
</tr>
<tr>
<td></td>
<td>10 µg</td>
<td>46.4±3.7</td>
<td>95.3±9.3</td>
</tr>
<tr>
<td></td>
<td>100 µg</td>
<td>52.6±4.4</td>
<td>271.2±3.3</td>
</tr>
<tr>
<td></td>
<td>1 mg</td>
<td>49.0±2.0</td>
<td>232.9±11.4</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.02 mg</td>
<td>42.2±3.3</td>
<td>96.8±8.8</td>
</tr>
<tr>
<td></td>
<td>0.05 mg</td>
<td>43.6±3.8</td>
<td>100.5±1.2</td>
</tr>
<tr>
<td></td>
<td>0.1 mg</td>
<td>44.3±0.7</td>
<td>100.7±2.0</td>
</tr>
<tr>
<td></td>
<td>0.2 mg</td>
<td>39.6±2.9</td>
<td>89.9±7.8</td>
</tr>
<tr>
<td></td>
<td>2.0 mg</td>
<td>50.0±3.3</td>
<td>101.6±3.3</td>
</tr>
<tr>
<td>SC 11800</td>
<td>0.01 mg</td>
<td>43.5±3.5</td>
<td>76.1±3.7</td>
</tr>
<tr>
<td></td>
<td>0.1 mg</td>
<td>55.0±5.3</td>
<td>190.9±50.6</td>
</tr>
<tr>
<td></td>
<td>0.5 mg</td>
<td>42.0±3.6</td>
<td>144.6±12.3</td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>47.5±3.4</td>
<td>159.3±15.6</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.1 mg</td>
<td>71.3±3.3</td>
<td>192.8±16.0</td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td>58.2±3.0</td>
<td>152.7±11.7</td>
</tr>
</tbody>
</table>

* Mean±s.e.
used for the purpose of oral contraception because of its strong gestagen action.

SC 11800 is odorless and white crystal which is soluble in oil. Elton (1961), Jones et al. (1966) and Fujimoto et al. (1966) determined with McPhail scale the degree of proliferation in the gland of the uterus of white young female rats treated with SC 11800 for several days. They reported that SC 11800 had a progesterone action in dose-response relation between the dose level and reaction. Such a progesterone-like action has also been confirmed with Clauberg test or carbonic anhydrase reaction. It was reported that a deciduogenetic action by the uterine trauma of experimental animals was reacted with a progesterone activity. Fujimoto et al. (1966) investigated three groups of female mice treated with SC 11800, SC 11800 plus mestranol, and mestranol, and found a deciduogenetic action only in groups treated with SC 11800 and SC 11800 plus mestranol to the same extent, but no changes in a group treated with mestranol. Accordingly, SC 11800 was clarified to have a progesterone-like action.

SC 11800 has also an estrogen-like action. Fujimoto et al. (1966) and Tokuda et al. (1967) reported, using the method of weighing mouse uterus, that SC 11800 had an estrogen-like action. Especially, Tokuda et al. (1967) confirmed its estrogen activity by the fact that cornified cells appeared in the vaginal smear of castrated female rats treated with more than 100 μg of SC 11800.

However, SC 11800 exhibited anti-progesterone and antiestrogen actions when it was administered in a large dose together with progesterone or estrogen. Consequently, it is considered that actions of SC 11800 take several aspects depending upon the state of hormones in the organism.

Progesterone is known to inhibit copulative ovulation in the rabbit, but there are no reports concerning its influences on estrous cycle in the female rat with normal estrous cycle. Progesterone is reported to cause pseudopregnancy when it is administered to the female rat with normal estrous cycle. Everett (1963) reported that pseudo-pregnancy revealed itself when progesterone was administered in dose of 2 mg for 5 days and 10 mg for a day. Rothchild et al. (1963) and Seike (1970) evaluated the rate of manifestation of pseudo-pregnancy when progesterone was administered in several doses during the different stages in estrous cycle. We could find that normal ovulatory estrous cycle was inhibited in proportion to the dose level together with continuous diestrus, which seems to be due to its progesterone-like action because we could not find such a phenomenon when estrogen was administered.

The contents of pituitary gonadotropin were also determined in order to analyze SC 11800 actions. Progesterone showed continuous diestrus together with changes in pituitary FSH, LH and prolactin when it was administered for a long time, and SC 11800 showed the same action. Drill (1963) stated that accumulation of hypophyseal gonadotropin was inhibited after castration in the rabbit treated with SC 11800.

There are some reports on the inhibiting action of SC 11800 against increase of pituitary gonadotropin after castration, but no reports on pituitary FSH, LH and
prolactin after treatment with SC 11800 for a long time in the rat with normal estrous cycle. Our results showed a decrease in LH in proportion to the amount of the drug administered and a tendency of increase in prolactin, but no marked changes in FSH. Since the above findings were more marked than when progesterone was administered and the reaction was different from that by estrogen or testosterone, it is considered that SC 11800 interferes with the pituitary gonadotropin secretion and its action is different from that of progesterone.

The pituitary gonadotropin secretion is inhibited by several synthetic gestagens, especially norethynodrel and norethindrone, and these influences on the ovary are not blocked after administration of gonadotropin to the rat pretreated in such a way. These gestagens seem to inhibit hypophyseal functions directly not via a level in the ovaries (Saunders and Drill 1958, Epstein et al. 1958), and SC 11800 is also considered to exhibit almost the same actions as these gestagens.

On the other hand, Stevens et al. (1965) reported that SC 11800 inhibited a peak of the LH appeared after the menstruation when it was administered to women at doses of 0.5, 1.0, and 2.0 mg. As we emphasized previously, it has been clarified that SC 11800, when employed in such a low dose that it could couple but pregnant and does not to change remarkably the estrous cycle, does not inhibit hypophyseal functions markedly.

Tokuda et al. (1967) found a relatively specific 32P uptake into the diencephalon and pituitary of the female rat with normal estrous cycle after treatment with SC 11800 for 30 days, and observed that 32P uptake into the hypophysis was lowered in proportion to the changes in ovarian weights. This indicates that SC 11800 acts on the diencephalon-pituitary system. However, Watanabe et al. (1968) pointed out that there was no tissue selectivity in 3H uptake into the organs of the female rat administered with 3H-labeled SC 11800. Therefore, the mechanism of inhibition of the diencephalon pituitary system by SC 11800 awaits further studies.

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References