EFFECTS OF EXOGENOUS OESTRADIOL AND PROGESTERONE ON SERUM LEVELS OF PROSTAGLANDIN F AND LUTEINIZING HORMONE IN CHRONICALLY OVARIECTOMIZED RATS

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(Received 7th August 1972)

Summary. Groups of rats ovariectomized 5 weeks previously were injected daily for 6 days with oestradiol and/or progesterone. Although the dose of oestradiol used was adequate to suppress peripheral serum LH values, none of the treatments significantly altered the peripheral serum levels of prostaglandin F.

Caldwell, Tillson, Brock & Speroff (1972) showed that in ovariectomized ewes injected with progesterone every 2 days over a 12-day period, followed 2 days later by a single injection of oestradiol, the prostaglandin F level in peripheral plasma rose significantly about 6 hr after the oestrogen treatment. Levels of prostaglandin F had returned to those seen before the oestrogen injection by 18 to 24 hr after the oestrogen treatment. In some ovariectomized animals, a slow rise of prostaglandin F was observed during the period of progesterone injections but a peak level was seen only after oestrogen stimulation. In addition, Blatchley, Donovan, Poyser, Horton, Thompson & Los (1971) and Blatchley, Donovan, Horton & Poyser (1972) have reported that daily injection of 10 µg oestradiol benzoate on Days 4 to 6 of the oestrous cycle of the guinea-pig caused elevated levels of prostaglandin F in the utero-ovarian venous plasma on Day 7. It has been postulated by Caldwell et al. (1972) that the rising levels of oestrogen on Day 13 of the oestrous cycle of the ewe are responsible for the production of prostaglandin F in the endometrium. This prostaglandin reaches the ovarian artery by the counter-current mechanism postulated by McCracken, Baird & Goding (1971) and causes a rapid fall in progesterone secretion on Days 13 to 14 of the cycle and demise of the corpus luteum.

We have therefore studied the effects of progesterone and oestrogen on the prostaglandin F levels in ovariectomized rats, and, for reference purposes concerning the effectiveness of the experimental treatments, have included measurements of LH levels in the same samples. Both progesterone and oestradiol have been found to inhibit or stimulate the secretion of LH depending on the dose, the time following ovariectomy, the time of day of steroid ad-

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ministration and collection of the blood sample, and the interval elapsing between steroid administration and blood collection (McCann, 1963; Caligaris, Astrada & Taleisnik, 1968).

Adult Sprague-Dawley virgin female rats, weighing 150 to 170 g (Charles River Breeding Laboratories), were housed in a temperature-controlled room lighted for only 14 hr a day. Unrestricted access was provided to food and water. The animals were bilaterally ovariectomized under Nembutal anaesthesia, and daily subcutaneous injections of 5 µg 17β-oestradiol (Sigma Chemical Co.) or 2 mg progesterone (Sigma Chemical Co.) were begun 35 days later. These steroids were dissolved in sesame oil in such a way that a constant injection volume of 0.2 ml/animal/day was used. The schedule of injections is shown in Table 1. All animals were killed by decapitation on the 7th day of the experiment (42 days after ovariectomy) and trunk blood was collected. All blood samples were allowed to clot at 4°C overnight and were centrifuged the next morning to obtain serum. The serum was frozen and stored at −20°C until assays were done.

**Table 1.** Experimental design for injection of groups of ovariectomized rats with progesterone or oestradiol

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td></td>
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<tr>
<td>II</td>
<td>P</td>
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<td>E</td>
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<td>IV</td>
<td>P</td>
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<td>V</td>
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P = progesterone, 2 mg/day subcutaneously. E = oestradiol, 5 µg/day subcutaneously. Day 1 = 36th day from ovariectomy and Day 7 = 42nd day from ovariectomy.

* Control animals; given 0·2 ml sesame oil/day.

For measurement of prostaglandin F (PGF) content, 1 ml serum, to which was added [3H]PGF$_{2\alpha}$ (~2000 d/min, New England Nuclear), was extracted as described by Skarnes & Harper (1972). The samples were fractionated on silicic-acid columns (Caldwell, Burstein, Brock & Speroff, 1971) and those containing PGF (average recovery 80%) were assayed in duplicate by radioimmunoassay (Stylos, Burstein, Rivetz, Gunsalus & Skarnes, 1972) using antisera raised in rabbits. Prostaglandin F$_{2\alpha}$ and PGF$_{1\alpha}$ compete in an identical manner with [3H]PGF$_2$ over a range of 0·1 to 2·5 ng of the assay. The values given are therefore referred to as PGF.

Serum LH was measured by radioimmunoassay using minor modifications of the system described by Niswender, Midgley, Monroe & Reichert (1968). Iodination was performed by a modification of the technique of Greenwood, Hunter & Glover (1952), as described by Scaramuzzi, Caldwell & Moor (1970). The LH standard, as determined by radioimmunoassay, was 0·055 times as potent as NIH-LH-S1. Serum LH levels were measured in 100-, 50- and 25-µl aliquots of the sample and all LH measurements were conducted in
the same assay. The Student t test was employed in evaluating the significance of differences between means.

Table 2 shows the levels of PGF and of LH found in the sera of the various experimental groups. The LH values presented are those from that aliquot sample which yielded an LH value on the same linear segment of the standard curve. Injections of 5 µg oestradiol for 6 days decreased the postcastration rise of LH to values near those for intact animals. Utilizing this LH standard, dioestrous plasma LH concentrations range between 30 and 50 ng/ml. Administration of progesterone alone did not significantly lower the serum LH concentration from that of ovariectomized controls, but the combination of oestradiol followed by progesterone was as effective in lowering LH levels as oestradiol alone. By contrast, when the progesterone treatment preceded that with oestradiol, a much smaller suppression of LH was observed (P<0.05).

None of the treatments used significantly changed the levels of PGF from that observed in ovariectomized controls.

<table>
<thead>
<tr>
<th>Exogenous steroid</th>
<th>PGF levels (ng/ml)</th>
<th>LH levels (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>(mean ± S.E.)</td>
<td>(mean ± S.E.)</td>
</tr>
<tr>
<td>Control</td>
<td>11.5±1.91 (5)*</td>
<td>740.8±143.3 (5)</td>
</tr>
<tr>
<td>Progesterone (P)</td>
<td>13.1±1.45 (5)</td>
<td>748.0±77.1 (5)</td>
</tr>
<tr>
<td>17β-oestradiol (E2)</td>
<td>11.4±0.74 (4)</td>
<td>967±28.2 (4)</td>
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<tr>
<td>P-E2</td>
<td>13.9±1.80 (5)</td>
<td>233.0±38.1 (5)</td>
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<tr>
<td>E2-P</td>
<td>15.2±1.64 (5)</td>
<td>92.8±20.3 (5)</td>
</tr>
</tbody>
</table>

* Figures in parentheses show the numbers of animals.

The ability of oestradiol to suppress serum or plasma LH levels of chronically ovariectomized rats to near those of intact animals is well documented (Gans, 1959; McCann & Taleisnik, 1961; Ramirez & McCann, 1963; Parlow, 1964; Caligaris et al., 1968). The failure of physiological doses of progesterone to suppress serum LH in such rats is in agreement with previous findings (McCann, 1962). Administration of progesterone following injections of oestradiol has been noted to suppress plasma LH levels to values below those found following administration of oestradiol alone (McCann, 1962). This response was obtained with injections of 4 mg progesterone/day, but not with injections of 1 mg/day. The use of 2 mg progesterone/day in the present study may account for the failure to observe a similar effect. The greater suppression of LH observed when progesterone was injected following oestradiol, compared to injection before oestradiol, is odd, but may be only a reflection of the time of sampling in relation to commencement of oestradiol injections rather than to the sequence in which the steroids were administered. The work of McCann & Taleisnik (1961) indicates that time of sampling after first injection of oestradiol is important in determining the resultant depression of plasma LH levels.

In view of the reports of Caldwell et al. (1972) and Blatchley et al. (1971) concerning elevated PGF levels following oestrogen treatment in sheep and guinea-pigs, respectively, the absence of such an effect in the present study is
perhaps surprising. However, the levels of PGF in these ovarietomized rats appear high (11.5 ng/ml). This is probably due to the fact that serum rather than plasma was used. Orczyk & Behrman (1972) have reported that the serum PGF level in immature female rats was 15.8 ± 2.3 ng/ml compared to plasma levels of only 1.4 ± 0.3 ng/ml. These authors concluded that this tenfold increase in PGF values was due to the contribution of PGF during the clotting process. Such high levels have not been observed in the sera of mice (Skarnes & Harper, 1972) or of humans (Editorial, 1972; Gutierrez-Cernosek, Zuckerman & Levine, 1972), also using a radioimmunoassay technique. However, a recent report by Silver, Smith, Ingerman & Kocsis (1972) showed that serum levels of PGF$_{2\alpha}$ and PGF$_{2\beta}$ in humans were increased if clotting was done at 37° C. The level of PGF in hamster serum appears to be about 10 ng/ml (S. K. Saksena and M. J. K. Harper, unpublished data) and is thus similar to that in rats. Levels of PGF measured in the plasma of rats throughout the oestrous cycle vary between 2.1 and 5.0 ng/ml of plasma (S. K. Saksena and M. J. K. Harper, unpublished data).

The present experiments had already commenced when this difference between serum and plasma levels of PGF in rats was reported. Thus, if oestrogen induced only a small change in peripheral plasma levels of PGF (say from 0.5 to 1 ng/ml, as observed by Caldwell et al., 1972 for sheep), such a difference might be masked by the high baseline values recorded in serum. In this connection, however, it should be noted that oestradiol alone in a serum PGF value identical to that of control ovarietomized animals. It would appear that neither oestrogen nor progesterone, alone or in combination at doses which effect serum LH levels, change peripheral PGF levels in chronically ovarietomized rats.

Thanks are due to Dr G. D. Niswender and Dr A. R. Midgley for the LH antiserum, to Dr L. E. Reichert, Jr for the LH standard and to Dr W. Stylos for the PGF antiserum. This study was supported by A.I.D. contract (csd/2837), the Ford Foundation (S.K.S.) and N.I.C.H.D. (R.S.) training programmes in Reproductive Physiology, and by a Career Development Award from N.I.C.H.D. (K4-HD-42, 369) to M.J.K.H.

REFERENCES


Oestradiol and prostaglandin F levels


