BLOCKADE OF THE SURGE OF PROLACTIN AT PRO-OESTRUS BY ADMINISTRATION OF ANTI-OESTROGENIC SUBSTANCE, MER-25

By
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ABSTRACT

A non-steroidal oestrogen antagonist, MER-25, was administered to cycling rats for elucidating the role of oestrogen in the surge of prolactin observed on the afternoon of pro-oestrus (POe).

In animals injected with 20 mg of MER-25 intramuscularly on the afternoon (16.30 h) of the first day of dioestrus (D-1), the surge of prolactin was blocked while the level of prolactin on the afternoon of POe of these animals was significantly higher than that of the corresponding controls injected with oil. Ovulation was also blocked in these animals treated with the drug on the afternoon of D-1. On the other hand, treatment on the morning (10.30 h) of the 2nd day of dioestrus failed to prevent not only the surge of prolactin but also ovulation.

These observations provide strong evidence for the view that oestrogen is responsible for the surge of prolactin on the afternoon of POe, and that the surge is accompanied by that of LH.

In a previous paper, we demonstrated that ovariectomy performed on the afternoon (around 16.30 h) of the 2nd day of dioestrus (D-2, day before pro-oestrus) in the rat with a 4-day cycle could prevent the surge of prolactin which occurred on the afternoon of pro-oestrus (POe) and that the administration of oestrogen at the time of ovariectomy could overcome the effect of ovariectomy on the surge. We suggested, therefore, that the surge of prolactin
could be triggered off by oestrogen secreted between D-2 afternoon and POE morning (Yokoyama et al. 1971b). Similar results were reported by Neill et al. (1971) and Freeman et al. (1972).

In order to elucidate further the role of oestrogen in regulating prolactin surge on the afternoon of POE, the effect of a non-steroidal anti-oestrogenic substance, MER-25 on the surge of prolactin was investigated.

Shireley et al. (1968) showed that administration of MER-25 could inhibit the occurrence of LH surge and prevent ovulation in the rat. Therefore, the effect of the drug on ovulation was also checked in the present experiments to see whether the inhibition of the surge of prolactin was accompanied by inhibition of the LH surge.

Part of this study was reported in a preliminary form (Yokoyama et al. 1972, in Japanese).

MATERIAL AND METHOD

Virgin female rats of the Wistar-Imamichi strain weighing 250–280 g were used. They were maintained under the same experimental conditions as described in the previous papers (Yokoyama et al. 1971a,b).

Vaginal smears were taken daily in the morning between 09.30–10.00 h. Only animals showing more than 2 consecutive, regular 4-day cycles were used.

Twenty mg of MER-25 (ethamoxytriptophol, Wm. S. Merrell Co.) was suspended in 0.5 ml of soya been oil and injected intramuscularly on the afternoon of the first day of dioestrus (D-1) or on the morning of D-2. In control animals, 0.5 ml of soya been oil was injected. Animals were decapitated under light ether anaesthesia on the afternoon (17.30 h − 18.00 h) of POE or on the morning (10.30 h − 11.00 h) of oestrus (Oe).

The anterior pituitaries, ovaries and uteri were removed and weighed on a torsion balance. The pituitary glands were stored frozen until assayed. Prolactin of the pituitary gland was fractionated by disc electrophoresis on polyacrylamide gel and measured by microdensitometer (Chromoscan, MK-II, Joyce Loeble Co. Ltd., England). The specificity and accuracy of the method used were reported by Yanai & Nagasawa (1969) and Nicoll et al. (1969). Since a linear relationship between the standard prolactin and their optical densities, obtained by these investigators, was confirmed in our laboratory by using NIH-P-B 3, the results are expressed as counts of the densitometer.

The occurrence of ovulation was determined by examination for the presence of ova in the oviducts of animals killed on the morning of Oe. The number of ova shed was counted under a microscope.

Significance of differences was calculated by Duncan’s multiple range test as modified by Kramer (1956).

RESULTS AND DISCUSSION

The levels of prolactin content and concentration in the pituitary gland in oil-injected control animals killed on the afternoon of POE were as low as
those reported previously in intact cyclic rats killed at the same stage of the cycle (Yokoyama et al. 1971a). In animals treated with MER-25 on the afternoon of D-1, the prolactin content and concentration on the afternoon of POe were significantly higher than those in the corresponding control animals (Table 1). On the other hand, the levels in animals treated with MER-25 on the morning of D-2 were not significantly different from those obtained in the controls.

Since the decrease in prolactin content and concentration in the pituitary gland on the afternoon of POe coincides very well with the elevation in the plasma prolactin levels reported recently by several investigators (Kwa & Verhofstad 1967; Neill 1970; Wuttke & Meites 1970), results of the present study clearly indicate that MER-25 administered on the afternoon of D-1 block the surge of prolactin which normally occurs on the afternoon of POe. The fact that the weights of both the uterus and the anterior pituitary gland in animals treated with MER-25 on D-1 were lower than those in the control animals killed at the same stages of the cycle indicates the anti-oestrogenic effect of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of autopsy</th>
<th>No. of rats</th>
<th>Anterior pituitary (AP) wt. mg</th>
<th>Prolactin[^4]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Content (counts/AP)</td>
</tr>
<tr>
<td>MER D-1</td>
<td>POe</td>
<td>5</td>
<td>9.44 ± 0.35[^ab]</td>
<td>498.85 ± 26.02[^d]</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>8.70 ± 0.39[^a]</td>
<td>573.65 ± 33.87[^de]</td>
</tr>
<tr>
<td>MER D-2</td>
<td>POe</td>
<td>5</td>
<td>9.32 ± 0.47[^ab]</td>
<td>289.20 ± 19.55[^f]</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>8.52 ± 0.16[^a]</td>
<td>579.40 ± 59.32[^de]</td>
</tr>
<tr>
<td>OIL D-1</td>
<td>POe</td>
<td>5</td>
<td>10.88 ± 0.26[^c]</td>
<td>388.51 ± 8.05[^f]</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>4</td>
<td>10.65 ± 0.55[^bc]</td>
<td>670.60 ± 49.86[^e]</td>
</tr>
<tr>
<td>OIL D-2</td>
<td>POe</td>
<td>5</td>
<td>10.28 ± 0.44[^bc]</td>
<td>353.60 ± 19.50[^f]</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>10.68 ± 0.51[^c]</td>
<td>857.10 ± 58.32[^e]</td>
</tr>
</tbody>
</table>

1: MER-25 (20 mg in 0.5 ml oil) or oil (0.5 ml) was injected intramuscularly on the afternoon (16.30 h) of the 1st day of dioestrus (D-1) or on the morning (10.30 h) of the 2nd day of dioestrus (D-2).
2: POe: pro-oestrus 17.30 h, Oe: oestrus 10.30 h.
3: Mean ± SEM.
4: Prolactin content and concentration are expressed as counts of the densitometer.
   a–l: Means which have the same superscripts are not significantly different from each other.
the drug used here (Table 2). Hence blockade of the surge of prolactin could be ascribed to the anti-oestrogenic effect of the drug. This anti-oestrogenic effect could also account for the lower level of prolactin in MER-25 treated animals on the morning of Oe than in the oil-treated control animals. A stimulating effect of oestrogen on the synthesis and release of prolactin has been well documented (Amenomori et al. 1970; Chen & Meites 1970).

The site of action of oestrogen for triggering off the surge of prolactin is still only conjectural. Direct action of oestrogen on the pituitary gland to stimulate the release of prolactin has been reported by Nicoll & Meites (1962). Recently, Valverde-R et al. (1972) reported that oestrogen increased the sensitivity of the anterior pituitary gland to the prolactin releasing factor. It can also be assumed that oestrogen decreases the response of the pituitary gland to prolactin inhibiting factor (PIF). On the other hand, there is some evidence to indicate an indirect action of oestrogen on the pituitary gland through the hypothalamus since PIF was markedly depleted after the administration of oestrogen in rats (Sar & Meites 1967). Implantation of oestrogen into the median eminence elevated the plasma level of prolactin (Nagasawa et al. 1969) and caused development of the mammary gland which was attributed to an increased secretion of prolactin (Ramirez & McCann 1964). According to the hypothesis of a dual hypothalamic control of prolactin proposed by Neill (1972), these results of the previous investigators could be explained by the stimulating effect of oestrogen on the centre for the tonic control of prolactin. The surge of prolactin, on the other hand, might be triggered off by the stimulating effect of oestrogen on the surge centre.

Only one out of 5 animals treated with MER-25 on the afternoon of D-1 ovulated. The number of ova shed in this animal was very small as compared to that of control animals or animals treated with MER-25 on the morning of D-2 (Table 2). Ovulation occurred in 4 out of 5 animals treated with MER-25 on the morning of D-2 and in all animals injected with oil. Therefore, the treatment which resulted in an inhibition of the prolactin surge could also prevent the surge of LH responsible for ovulation. These results on the blockade of ovulation by MER-25 were essentially the same as those reported by Shireley et al. (1968), although MER-25 had to be injected in order to block ovulation 17 hours earlier in the present study than in the experiment of Shireley et al. (1968). This discrepancy may be ascribed to the dosage of the drug (20 mg in the present experiments vs. 40 mg) and the strain of rats used (Wistar-Imamichi vs. Sprague-Dawley).

It has been reported (Yokoyama et al. 1971a; Wuttke & Meites 1970) that the surge of prolactin as well as that of LH was prevented by pentobarbitone injected just before the ‘critical period’ for LH. In the present study we have demonstrated that an anti-oestrogenic drug prevents not only the surge of prolactin but also that of LH. These findings seem to imply that the surge of
Table 2.
Effects of MER-25 (ethamoxytriphetol) treatment on ovulation, number of ova and on weight of ovaries and uterus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of autopsy</th>
<th>No. of rats</th>
<th>Ovarian weight mg</th>
<th>No. of rats ovulated</th>
<th>Avg. No. of ova shed</th>
<th>Uterine weight mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MER D-1</td>
<td>POe</td>
<td>5</td>
<td>68.22 ± 3.283a</td>
<td></td>
<td></td>
<td>303.76 ± 25.29ef</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>77.66 ± 5.09ab</td>
<td>1/5</td>
<td>6</td>
<td>276.14 ± 23.16f</td>
</tr>
<tr>
<td>MER D-2</td>
<td>POe</td>
<td>5</td>
<td>79.36 ± 3.95ab</td>
<td></td>
<td></td>
<td>319.24 ± 11.73ef</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>80.40 ± 5.27ab</td>
<td>4/5</td>
<td>13.0 ± 1.54</td>
<td>330.54 ± 16.28def</td>
</tr>
<tr>
<td>OIL D-1</td>
<td>POe</td>
<td>5</td>
<td>87.46 ± 3.46b</td>
<td></td>
<td></td>
<td>454.72 ± 14.25c</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>4</td>
<td>87.77 ± 9.53b</td>
<td>4/4</td>
<td>14.3 ± 1.3</td>
<td>348.15 ± 13.97de</td>
</tr>
<tr>
<td>OIL D-2</td>
<td>POe</td>
<td>5</td>
<td>80.08 ± 4.28ab</td>
<td></td>
<td></td>
<td>483.24 ± 16.81c</td>
</tr>
<tr>
<td></td>
<td>Oe</td>
<td>5</td>
<td>88.72 ± 2.29b</td>
<td>5/5</td>
<td>13.8 ± 1.1</td>
<td>385.60 ± 15.08d</td>
</tr>
</tbody>
</table>

1: MER-25 (20 mg in 0.5 ml oil) or oil (0.5 ml) was injected intramuscularly on the afternoon (16.30 h) of the 1st day of dioestrous (D-1) or on the morning (10.30 h) of the 2nd day of dioestrous (D-2).

2: POe: pro-oestrus 17.30 h, Oe: oestrus 10.30 h.

3: Mean ± SEM.

4: One rat showing no ovulation was excluded for calculation of the mean.

a-f: Means which have the same superscripts are not significantly different from each other.

both prolactin and LH is regulated at least partly by a common mechanism. Further support for the existence of a common mechanism seems to be provided by experiments carried out by Uchida et al. (1972), in which they demonstrated that not only the release of LH but also that of prolactin was advanced by the administration of progesterone on the morning of POe in the rat. However, Everett & Quinn (1966) demonstrated the differential hypothalamic control of the release of LH and prolactin. In addition, electrochemical stimulation of the pre-optic area in the rat, where the surge of LH was believed to be controlled (Gorski 1968), failed to increase the plasma level of prolactin (Karla et al. 1971; Fukushima & Yokoyama, unpublished data). Further studies are required to clarify the hypothalamic area controlling the surge of prolactin.

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REFERENCES


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