THE EFFECT OF HYPOTHALAMIC STIMULATION AND PROGESTERONE ON OVULATION IN FEMALE RATS TREATED WITH THE OESTROGEN ANTAGONIST ICI 46,474

By

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ABSTRACT

A single dose (0.2 mg) of the antioestrogen ICI 46,474 at 15:00 h on the second day of dioestrus inhibited ovulation in the female rat. This inhibition could not be overcome by electrochemical stimulation of the basal hypothalamus on the afternoon of pro-oestrus. In contrast, progesterone (1.0 mg) treatment at 10:30 h on pro-oestrus induced ovulation at the expected time. The facilitatory effect of progesterone was prevented by administration of Nembutal (35 mg/kg) at 11:00 h on pro-oestrus. Progesterone did not induce ovulation in rats treated with ICI 46,474 on both dioestrus day 1 and 2. Sexual receptivity in ovariectomized oestradiol-treated (2.0 µg/day) rats was significantly depressed by the antioestrogen (1.0 mg/day) whether it was given 1 h before, 1 h after, or at the same time as the oestradiol. The compound also prevented the uterine weight increase in response to oestradiol but exerted a significant effect on uterine weight itself when compared to oil treated controls.

Ovulation may be inhibited by preventing the positive feedback effect of oestradiol on the ovulatory surge of gonadotrophin. Shirley et al. (1968) found that suitably timed injections of the antioestrogen MER 25 could prevent uterine ballooning, vaginal cornification and ovulation in the rat. More recently Labhsetwar (1970a,b) demonstrated similar effects using ICI 46,474.
Ferin et al. (1969a,b) showed that antibodies raised against 17β-oestradiol could prevent ovulation in both PMS-treated immature rats and adult cycling animals. Intracranial implants of the antioestrogen ICI 46,474 suggest that it exerts its effects via the basal hypothalamus and/or anterior pituitary (Bainbridge & Labhsetwar 1971; Billard & McDonald, in press). In view of its central action it was considered of interest to study the effects of hypothalamic stimulation and progesterone treatment in rats receiving ovulation-inhibiting doses of ICI 46,474. In addition its effects on oestradiol-induced lordosis behaviour were investigated.

METHODS

Adult virgin female rats of the Sprague-Dawley strain were housed in a light and temperature controlled room. Lights were on from 05:00 h to 19:00 h. Vaginal smears were taken daily and only those rats with a minimum of two consecutive 4-day cycles were used for the experiments. Electrochemical stimulation of the basal hypothalamus was carried out on the afternoon of pro-oestrus under Nembutal anaesthesia. Details of the electrodes and stimulation procedure used have been described previously (McDonald & Gilmore 1971a). The antioestrogen (ICI 46,474) was dissolved in corn oil and administered subcutaneously at a level of either 0.2 mg or 1.0 mg in 0.1 ml corn oil. Oestradiol benzoate was similarly administered at a dose of 2.0 μg in 0.1 ml. Animals were normally autopsied on the day of expected oestrus. The Fallopian tubes were examined and the number of ova present counted. The weights of the ovaries and uteri were recorded. Brains from rats which had received hypothalamic stimulation were perfused with a 3% w/v solution of potassium ferro/ferri cyanide to develop the Prussian blue reaction. Frozen sections were taken and stained with thionin and the position of the electrode verified histologically. For behaviour studies, rats were bilaterally ovariectomized under ether anaesthesia. Two weeks later they were given 7 daily doses of 2 μg oestradiol benzoate either 1 h before, 1 h after, or at the same time as 1.0 mg of antioestrogen. Control groups of animals received oil, oestradiol benzoate or antioestrogen alone. Testing took place on the last five days of treatment in semi-circular glass fronted cages during the dark phase of a reversed day/night cycle. The number of mounts and lordoses were recorded and the lordosis quotient (L/M x 100) calculated.

The experimental results were analysed using Student's t-test and Fisher's exact probability test (Siegel 1956).

RESULTS

In preliminary studies 0.2 mg was found to be the minimum dose of antioestrogen which would effectively delay ovulation for a minimum of 24 h when given at 15:00 h on the second day of dioestrus. Of 13 rats given this dose (Table 1 Group 1), 4 were delayed for 24 h and the remaining 9 for longer than 24 h. Stimulation of the medial basal hypothalamus in the region
The effect of hypothalamic stimulation and progesterone on ovulation in rats treated with the antioestrogen ICI 46,474. Mean values ± sem are given.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Time</th>
<th>No. of rats</th>
<th>No. ovulating</th>
<th>No. of ova per ovulating rat</th>
<th>Ovarian wt. (mg)</th>
<th>Uterine wt. (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ICI 46,474</td>
<td>15:00</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>60.7 ± 8.7</td>
<td>354.8 ± 22.6</td>
</tr>
<tr>
<td>2</td>
<td>ICI 46,474</td>
<td>15:00</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>78.0 ± 3.6</td>
<td>373.9 ± 13.8</td>
</tr>
<tr>
<td></td>
<td>HYP STIM</td>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>HYP STIM</td>
<td>14:00</td>
<td>5</td>
<td>4</td>
<td>11.5 ± 1.5</td>
<td>73.6 ± 10.1</td>
<td>397.2 ± 25.9</td>
</tr>
<tr>
<td>4</td>
<td>ICI 46,474</td>
<td>15:00</td>
<td>15</td>
<td>15</td>
<td>10.8 ± 0.5</td>
<td>72.5 ± 3.4</td>
<td>346.7 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>PROG</td>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>ICI 46,474</td>
<td>15:00</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>58.9 ± 3.1</td>
<td>312.4 ± 25.7</td>
</tr>
<tr>
<td></td>
<td>ICI 46,474</td>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PROG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ICI 46,474</td>
<td>15:00</td>
<td>5</td>
<td>1</td>
<td>9</td>
<td>62.6 ± 5.5</td>
<td>320.0 ± 17.2</td>
</tr>
<tr>
<td></td>
<td>PROG</td>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D1, dioestrus day 1. D2, dioestrus day 2. Pe, pro-oestrus.
HYP STIM, hypothalamic stimulation 100 µA, 60 sec.
ICI 46,474 0.2 mg. PROG, progesterone 1.0 mg.
PB, sodium pentobarbitone 35 mg/mg.

of the arcuate nucleus induced ovulation in only two animals treated with 0.2 mg antioestrogen 24 h previously (Group 2), in contrast ¼ control rats ovulated a normal number of eggs to the same stimulus parameters (Group 3).

The inability of median eminence stimulation to induce ovulation prompted us to investigate whether progesterone treatment on the day of pro-oestrus would overcome the inhibition. Animals receiving 0.2 mg ICI 46,474 on the second day of dioestrus all ovulated at the expected time when treated with progesterone at 10:30 h on pro-oestrus (Group 4). However, when the antioestrogen was given on both dioestrus day 1 and 2, progesterone failed to
The effect of ICI 46,474 on the uterine weight and lordosis quotient in ovariectomized oestradiol-treated rats.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Uterine weight (mg) mean ± sem</th>
<th>L. M.</th>
<th>L. Q.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oil</td>
<td>5</td>
<td>195 ± 16.3</td>
<td>0/250</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>ICI</td>
<td>5</td>
<td>100 ± 11.5^a)</td>
<td>6/260</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>OB</td>
<td>10</td>
<td>380.2 ± 25.2^b)</td>
<td>217/500</td>
<td>43.4</td>
</tr>
<tr>
<td>4</td>
<td>ICI + OB 60 min later</td>
<td>10</td>
<td>211.8 ± 11.2^a)</td>
<td>57/450^c)</td>
<td>12.7</td>
</tr>
<tr>
<td>5</td>
<td>ICI + OB 0 min later</td>
<td>5</td>
<td>204.2 ± 22.8^a)</td>
<td>52/250^c)</td>
<td>20.8</td>
</tr>
<tr>
<td>6</td>
<td>OB + ICI 60 min later</td>
<td>5</td>
<td>195.4 ± 27.6^a)</td>
<td>26/250^c)</td>
<td>10.4</td>
</tr>
</tbody>
</table>

ICI 46,474 1.0 mg daily. OB, oestradiol benzoate 2.0 µg daily.
L. Q., lordosis quotient. L/M, lordosis/mount.
^a) P < 0.01 vs groups 1,3
^b) P < 0.01 vs groups 1,2,4,5,6
^c) P < 0.05 vs groups 1,2,3

Induce ovulation (Group 5). In order to determine if progesterone might be acting directly on the anterior pituitary, rats receiving the antioestrogen were given Nembutal 30 min after the progesterone. Only 1 out of 5 rats ovulated at the expected time (Group 6).

The results in Table 2 show that the antioestrogen significantly depresses the lordosis response in ovariectomised oestrogen-treated females. The effect was equally noticeable whether the compound was given 1 h before, 1 h after or at the same time as the oestradiol. When given alone the antioestrogen had no significant effect on behaviour. Although the uterine weight was significantly increased by the antioestrogen it clearly prevented any additional increase in weight due to oestradiol treatment.

**DISCUSSION**

The inhibition of ovulation following the subcutaneous administration of ICI 46,474 confirms the previous observations of Labhsetwar (1970a,b) and further supports the hypothesis that the preovulatory rise in oestradiol levels is a prerequisite for the occurrence of ovulation. In previous studies in which ovula-
tion was prevented or delayed by the administration of progesterone or nor-
ethindrone (McDonald & Gilmore 1971a,b) it was possible to overcome the
inhibition by stimulation of either the preoptic area or basal hypothalamus.
In the present experiments the basal hypothalamus was unresponsive to electro-
chemical stimulation. Even the two rats which ovulated had only a single egg
each. This finding is surprising since the stimulation parameters used for both
groups 2 and 3 were some four times the threshold required in these rats
(McDonald & Gilmore 1971a). These findings could be due to the inability of
the anterior pituitary to respond to LH-RH. However, Labhsetwar (1970a,b)
has shown previously that treatment with ICI 46,474 does not affect the
response of the pituitary to exogenous LH-RH. Furthermore the ability of
progesterone to restore ovulation also suggests that the sensitivity of the ante-
rior pituitary gland to releasing hormone is not involved. Since the facilitatory
response to progesterone was prevented by treatment with Nembutal it in-
dicates that the pituitary is not the site of action of progesterone. This latter
finding is of some significance since it suggests that the neurons of the basal
hypothalamus are differentially sensitive to electrochemical stimulation and
progesterone in the presence of an antioestrogen. The mechanism by which this
is brought about is not known at present. However, the response to progester-
one appears to require some oestrogen conditioning since treatment with anti-
oestrogen on both dioestrus day 1 and 2 prevented the facilitatory effect of
progesterone. This confirms the previous observations of Caligaris et al. (1968,
1971) who also showed the necessity for oestrogen conditioning before pro-
gesterone would induce the release of gonadotrophins.

The uterine weight data in Table 2 clearly show that ICI 46,474 possesses
inherent oestrogenic activity since it significantly increased uterine weight over
the oil treated controls. However, it is equally clear that the action of oestra-
diol was completely blocked by the antioestrogen. The compound also inhibited
oestradiol induced receptivity in the female and was completely ineffective on
its own. Thus, although the inherent oestrogenic properties of the compound
were sufficient to influence uterine weight the less sensitive neural mechanism
was not activated. This agrees with the observations of Davidson et al. (1968)
who showed that uterine weight was more sensitive to oestrogenic stimulation
than was receptivity. It is interesting that when both oestradiol and the antio-
oestrogen were given together the females exhibited a higher, though not
significantly so, degree of receptivity compared to treatment with the antio-
oestrogen before or after the oestradiol. There is no obvious explanation for
this finding at present.
ACKNOWLEDGMENTS

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