EVIDENCE FOR A DOPAMINERGIC COMPONENT IN THE SERIES OF NEURAL EVENTS THAT LEAD TO THE PRO-OESTROUS SURGE OF LH

By

James A. Clemens, Frank C. Tinsley and Ray W. Fuller

ABSTRACT

The possible participation of dopamine in the neural events that lead to the pro-oestrous surge of luteinizing hormone (LH) was investigated utilizing a dopaminergic ergoline derivative (lergotrile mesylate). Administration of reserpine (2.0 mg/kg, ip) to rats on the day of pro-oestrus depleted brain dopamine and norepinephrine and prevented the LH surge and ovulation. Administration of lergotrile mesylate prior to or at the same time as reserpine prevented the inhibitory effects of reserpine on LH release and on ovulation in about half of the animals. When lergotrile mesylate was given on the morning of pro-oestrus, the LH surge was advanced. The results indicate that there is a dopaminergic component in the series of neural events that precede the surge of LH on pro-oestrus, and that the dopaminergic stimulus precedes the LH surge by about 4–5 h.

Monoaminergic participation in the control of the pro-oestrous surge of luteinizing hormone (LH) has been well established. The precise role of dopamine, however, is not clear. Earlier reports have suggested that dopamine is stimulatory (Schneider & McCann 1970; Kamberi et al. 1970), inhibitory (Fuxe et al. 1973; Sawyer et al. 1974) or has no effect (Craven & McDonald 1971) on LH release. Most of the above experiments were performed under different experimental conditions and that perhaps is why conflicting results were obtained.

Recent studies have shown that some ergot derivatives are dopamine receptor...
stimulants (Corrodi et al. 1973). Interestingly, it was observed several years ago that when women with the amenorrhoea-galactorrhoea syndrome were treated with bromocryptine not only did lactation cease but regular menstrual cycles accompanied by ovulation began (Pozo et al. 1972). Prolactin may have an inhibitory influence on the ovary in the women with the amenorrhoea-galactorrhoea syndrome. In the rat a condition similar to the amenorrhoea-galactorrhoea syndrome can be produced by reserpine. This condition is characterized by elevated serum prolactin levels, inhibition of LH secretion and blockade of ovulation. In the present study we examined the influence of the dopaminergic ergoline derivative, lergotrile mesylate (Clemens et al. 1975), on LH release and ovulation in reserpine-treated rats.

MATERIALS AND METHODS

Animals

Mature Sprague-Dawley female rats were used in this study. The rats were housed in a temperature (22–25°C) and light (lights on 06.50–18.50 h) controlled room. The rats were fed lab chow and tap water ad libitum. Vaginal smears were recorded daily.

Effects of reserpine and lergotrile mesylate on ovulation

Rats were selected for experimentation on the day of pro-oestrus. Reserpine (Sandril®, Eli Lilly) or lergotrile mesylate were given by injection (subcutaneously) either alone or in combination at various times of day on the day of pro-oestrus. The rats were killed on the day of oestrus, and the oviducts were removed and searched for ova by microscopic examination. The influence of reserpine on brain norepinephrine and dopamine levels was determined in rats receiving reserpine (2 mg/kg sc) at 10.00 h on pro-oestrus and killed at 15.50 h. Dopamine and norepinephrine were assayed by the method of Chang (1964). This provided a measure of brain norepinephrine and dopamine levels during the time that the ovulatory surge of luteinizing hormone normally occurs and assured us that the reserpine-treatment actually was reducing brain catecholamines.

Effects of lergotrile mesylate and reserpine on serum luteinizing hormone levels

Rats were selected for experimentation on the day of pro-oestrus. In one part of the study we examined the influence of lergotrile mesylate on LH release. Lergotrile mesylate was injected sc (4.0 mg/kg) at 09.50 h. One group of rats was killed at 13.50 h and another group was killed at 14.25 h. Control rats injected with vehicle (sterile water) were killed at similar times. After decapitation the blood was collected for radioimmunoassay of LH. In another part of the study we examined the influence of reserpine and lergotrile mesylate on LH release. One group of pro-oestrous rats received a vehicle injection and served as controls. A second group of rats received a subcutaneous injection of lergotrile mesylate (4 mg/kg) at 09.50 h and a subcutaneous injection of 2.0 mg/kg of reserpine at 13.50 h. In a third group of rats sterile water was injected at 09.50 and 2.0 mg/kg of reserpine was injected at 13.50 h. At 15.50 h the rats were decapitated and blood was collected for LH radioimmunoassay.
Luteinizing hormone radioimmunoassay

After the blood was collected it was allowed to clot at 4°C and then centrifuged at 1200 x g for 20 min. The serum was removed and assayed for LH by radioimmunoassay using the method of Niswender et al. (1968). LH was expressed as ng of LER1213A per ml of serum. LER1213A potency is 0.25 x NIAMD-LH-RP1. Serum levels of LH at times other than the pro-oestrous surge are generally in the range of 15–25 ng/ml. Since we were interested in whether the animals showed a pro-oestrous surge or not we selected the value of 50 ng/ml to be the serum level that needed to be attained to conclude that the rat was demonstrating a pro-oestrous surge. A value of 50 ng/ml is most probably at the beginning of the surge whereas values of 300 ng/ml or greater are observed as the surge advances.

RESULTS

The effects of reserpine alone and lergotrile mesylate alone on ovulation are shown in Table 1. Reserpine was able to block ovulation when given at several different times of day. The 2.0 mg/kg dose was more effective than lower doses. Lergotrile mesylate did not block ovulation. Rats treated with lergo-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of administration (h)</th>
<th>No. rats ovulating/No. rats in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reserpine, 1.2 mg/kg</td>
<td>08.00</td>
<td>2/4</td>
</tr>
<tr>
<td>Reserpine, 2.0 mg/kg</td>
<td>07.50</td>
<td>0/3</td>
</tr>
<tr>
<td>Reserpine, 1.0 mg/kg</td>
<td>10.00</td>
<td>2/3</td>
</tr>
<tr>
<td>Reserpine, 1.2 mg/kg</td>
<td>10.00</td>
<td>1/3</td>
</tr>
<tr>
<td>Reserpine, 2.0 mg/kg</td>
<td>10.00</td>
<td>0/15</td>
</tr>
<tr>
<td>Reserpine, 2.0 mg/kg</td>
<td>13.50</td>
<td>1/8</td>
</tr>
<tr>
<td>Reserpine, 2.0 mg/kg</td>
<td>15.00</td>
<td>2/5</td>
</tr>
<tr>
<td>Lergotrile mesylate, 2.0 mg/kg</td>
<td>10.00</td>
<td>2/2</td>
</tr>
<tr>
<td>Lergotrile mesylate, 4.0 mg/kg</td>
<td>08.00</td>
<td>8/8</td>
</tr>
<tr>
<td>Control, vehicle</td>
<td>08.00</td>
<td>7/7</td>
</tr>
<tr>
<td>Control, vehicle</td>
<td>10.00</td>
<td>14/15</td>
</tr>
<tr>
<td>Control, vehicle</td>
<td>12.50</td>
<td>5/5</td>
</tr>
</tbody>
</table>
**Table 2.**
Effects of lergotrile mesylate on the ability of reserpine to block ovulation.

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Time of treatments (h)</th>
<th>No. rats ovulating/total No.</th>
</tr>
</thead>
</table>
| 1. Reserpine, 2 mg/kg  
Lergotrile mesylate, 2 mg/kg | 10.00  
08.00 | 1/5 |
| 2. Reserpine, 2 mg/kg  
Lergotrile mesylate, 4 mg/kg | 10.00  
08.00 | 4/7 |
| 3. Reserpine, 2 mg/kg  
Lergotrile mesylate, 4 mg/kg | 08.00  
10.00 | 0/5 |
| 4. Reserpine, 2 mg/kg  
Lergotrile mesylate, 2 mg/kg | 10.00  
10.00 | 4/10 |
| 5. Reserpine, 2 mg/kg  
Lergotrile mesylate, 4 mg/kg | 10.00  
10.00 | 4/8 |
| 6. Reserpine, 2 mg/kg  
Lergotrile mesylate, 4 mg/kg | 13.50  
09.50 | 7/8 |

**Table 3.**
Effects of reserpine on brain catecholamine levels in pro-oestrous rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Norepinephrine ( \mu g/g ) brain</th>
<th>Dopamine ( \mu g/g ) brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, vehicle (6)</td>
<td>(0.48 \pm 0.01^{*})</td>
<td>(1.52 \pm 0.03)</td>
</tr>
<tr>
<td>Reserpine, 2 mg/kg (6)</td>
<td>(0.11 \pm 0.01) ((P &lt; 0.001))</td>
<td>(0.72 \pm 0.02) ((P &lt; 0.001))</td>
</tr>
</tbody>
</table>

* Mean ± standard error.  
( ) = Number of rats.
trile mesylate ovulated an average of 13.5 eggs, and control groups ovulated an average of 11.8 eggs. Table 2 shows the influence of lergotrile mesylate on the ability of reserpine to block ovulation. Lergotrile mesylate was effective in preventing the inhibitory effects of reserpine on ovulation when administered before or at the same time as the reserpine. When lergotrile was given 2 h or more after the reserpine it was not able to appreciably prevent the inhibitory influence of reserpine. The effects of reserpine on dopamine and norepinephrine levels in whole brain are shown in Table 3.

**Effects of lergotrile mesylate and reserpine on the pro-oestrous surge of luteinizing hormone**

Table 4 shows that administration of lergotrile mesylate on the morning of pro-oestrous induced early surges of luteinizing hormone in 5/6 animals. When lergotrile mesylate was given at 09.50 h and the rats killed at 14.25 h, 1 rat out of 6 had an LH level < 50 ng/ml while 5/6 had LH levels > 50 ng/ml, and 3 of those 5 had levels > 100, and 1 of the 3 had levels > 300 ng/ml. Control animals did not show any evidence of LH surges. After administration of lergotrile mesylate on the morning of pro-oestrous, early surges of luteinizing hormone were observed in 5/6 animals. On the other hand, when lergotrile mesylate was given at 09.50 h and rats killed at 14.25 h, 1 rat out of 6 had an LH level < 50 ng/ml, while 5/6 had LH levels > 50 ng/ml, and 3 of those 5 had levels > 100, and 1 of the 3 had levels > 300 ng/ml. Control animals did not show any evidence of LH surges. After administration of lergotrile mesylate on the morning of pro-oestrous, early surges of luteinizing hormone were observed in 5/6 animals. On the other hand, when lergotrile mesylate was given at 09.50 h and rats killed at 14.25 h, 1 rat out of 6 had an LH level < 50 ng/ml, while 5/6 had LH levels > 50 ng/ml, and 3 of those 5 had levels > 100, and 1 of the 3 had levels > 300 ng/ml. Control animals did not show any evidence of LH surges.

**Table 4.** Effects of lergotrile mesylate and reserpine on the pro-oestrous surge of luteinizing hormone.

<table>
<thead>
<tr>
<th>Treatment and time</th>
<th>No. of rats</th>
<th>Time of sacrifice (h)</th>
<th>Serum LH levels (ng LER 1213A/ml)</th>
<th>&lt; 50</th>
<th>&gt; 50</th>
<th>&gt; 100</th>
<th>&gt; 200</th>
<th>&gt; 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lergotrile mesylate (4 mg/kg 09.50 h)</td>
<td>7</td>
<td>13.50</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Control, vehicle (09.50 h)</td>
<td>6</td>
<td>13.50</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lergotrile mesylate (4 mg/kg 09.50 h)</td>
<td>6</td>
<td>14.25</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Control, vehicle (09.50 h)</td>
<td>7</td>
<td>14.25</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lergotrile mesylate (4 mg/kg 09.50 h) + Reserpine (2 mg/kg 13.50 h)</td>
<td>6</td>
<td>15.50</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Control, vehicle (09.50 h) + Reserpine (2 mg/kg 13.50 h)</td>
<td>6</td>
<td>15.50</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Control, vehicle (09.50 h)</td>
<td>5</td>
<td>15.50</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
of reserpine (2.0 mg/kg) at 13.50 h only 2/6 rats showed surges of LH, while 5/6 rats treated with lergotrile mesylate (4 mg/kg) at 09.50 h and reserpine at 13.50 h showed LH surges at 15.50 h.

**DISCUSSION**

The results of this study indicate that lergotrile mesylate is able to advance the surge of LH and prevent the inhibitory effects of reserpine on LH release and ovulation in rats. This effect may occur because lergotrile mesylate stimulates hypothalamic dopamine receptors that are involved in LH releasing hormone secretion. We have previously reported that lergotrile mesylate inhibits prolactin release by virtue of its dopamine receptor stimulating properties (Clemens et al. 1975). Lergotrile mesylate also is able to induce turning behaviour typical of dopamine receptor stimulants in rats with unilateral lesions of the nigrostriatal tract produced with 6-hydroxydopamine (unpublished observations). Its ability to stimulate dopamine receptors probably explains why lergotrile mesylate is beneficial in treating Parkinson's disease (Lieberman et al. 1975). The ability of lergotrile mesylate to prevent the inhibitory effects of reserpine on the LH surge on pro-oestrous provides evidence that lergotrile mesylate can actively replace the dopamine which is depleted by reserpine. Norepinephrine and serotonin also are depleted by reserpine; however, lergotrile mesylate possesses no noradrenergic or serotoninergic properties. Most of the previous studies on the role of dopamine in the control of LH involve the use of anaesthetized animals and the injection of dopamine into the brain in relatively large amounts. The present study differs from previous ones in that we are depleting endogenous dopamine with reserpine and replacing the dopamine by systemic administration of a dopamine agonist.

The relative ineffectiveness of lergotrile mesylate when given 2 h after reserpine in reversing reserpine's inhibitory effects on ovulation may mean that reserpine is depleting some other monoamine necessary for LH release to occur. Perhaps a noradrenergic or serotoninergic neuron is interposed in a chain of neurons between the dopamine neurons and the LH releasing hormone elements. Another alternative is that some non-specific depressant effect of the reserpine was able to alter neural events necessary for the pro-oestrous LH surge, because a variety of anaesthetics, sedatives and tranquilizers are able to block ovulation in the rat. Regardless of what transmitter substance might be involved the results do indicate that reserpine is able to block some necessary event that occurs after the dopaminergic stimulus.

Therefore, we conclude that a dopaminergic stimulus precedes the LH surge by approximately 4 or 5 h. In addition we also agree with the findings of Fuxe et al. (1973) and Sawyer et al. (1974) that dopaminergic stimuli at about
the time LH is to be released blocks the surge of LH and ovulation. We also have observed that dopamine agonists block LH release when given shortly prior to the surge (unpublished observations). Thus the effects on LH release obtained after administration of dopamine agonists largely depend on the time of administration of the drug. Evidently there are two sets of dopaminergic neurons controlling LH release; one is stimulatory, and the other is inhibitory.

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REFERENCES


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