HYPOTHALAMO-HYPOPHYSIAL CONTROL OF OVULATION IN THE VOLE (MICROTUS AGRESTIS)

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Summary. Electrical stimulation of the anterior hypothalamus of the vole, Microtus agrestis, usually results in ovulation, whereas stimulation of areas in the posterior hypothalamus do not. Ovulation can also be elicited by injections of sheep median-eminence extracts, and by preparations of sheep luteinizing hormone. Observations on the number of corpora lutea formed, and ripe Graafian follicles remaining after treatment, suggest that mating is the most potent stimulus for ovulation, followed by electrical stimulation and hormone injection.

INTRODUCTION

The vole, Microtus agrestis, is a reflex ovulator (Austin, 1957; Breed, 1967) and ovulates about 10 hr after coitus (Breed & Clarke, 1970) or administration of pregnancy urine gonadotrophin (Austin, 1957). The effects of different daylight regimens on hypothalamic and pituitary histology have been investigated by Clarke & Kennedy (1967), but only one preliminary report on the hypothalamo-hypophysial control of ovulation has been published (Breed & Charlton, 1968). The present study, which is an elaboration and extension of the previously reported work, has been approached in three ways: (1) electrical stimulation of different regions of the hypothalamus, (2) intravenous injections of extracts of sheep median eminence into the suborbital canthal sinus, and (3) injections of different doses of luteinizing hormone (LH) both intravenously (i.v.) and intraperitoneally (i.p.).

MATERIALS AND METHODS

Perforate, adult, virgin, female voles were used in all experiments. The animals were killed 24 hr after treatment and the ovaries were serially sectioned at 7 µm and subsequently stained with Ehrlich's haematoxylin and eosin. A positive ovarian response was defined either by the presence of corpora lutea, or by the presence of stimulated follicles, which included luteinized follicles, follicles with strongly staining eosinophilic material and corpora lutea (see Everett, 1965).

Hypothalamic stimulation

Voles were anaesthetized with sodium pentobarbital and placed in a rat

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stereotaxic machine. A unipolar platinum electrode, encased in glass tubing with an exposed tip of 0·5 mm, was inserted in the mid-line into the brain through a hole drilled in the vault of the skull. The position of its tip in the hypothalamus was monitored by X-ray photography enabling the anterior/ posterior and dorso-ventral positions of the electrode to be determined. This was later confirmed by histology. Anterior, mid- and posterior hypothalamic areas were stimulated; these included the area posterior to the optic chiasma, the posterior median eminence and just anterior to the mamillary bodies. The current was passed in the form of a 50-cycle sine wave a.c., with an intensity of 200 microamps root mean square. The duration of stimulus was 15 min, current being delivered for 15 sec on, 15 sec off during this period. During stimulation the animal’s behaviour was observed and recorded.

Median-eminence extract administration

An extract of sheep hypothalami purified to Stage 3 as described by Gregory, Walpole, Charlton, Harris & Reed (1968) was administered i.v. through the suborbital canthal sinus. Fourteen of the eighteen experimental animals received this extract, four received material from Stage 1.

Luteinizing hormone administration

Varying dose levels of NIH-LH-s6, s7 or s11 were made up in saline and injected—0·07 to 5·0 µg i.p., or 0·01 to 2·0 µg i.v. into the canthal sinus.

RESULTS

Hypothalamic stimulation

Rotary movements of the eyeballs indicate spread of current to myelinated fibres of the oculomotor nerve (Harris, 1955). This was usually observed after stimulation of the anterior hypothalamus but only once after mid- and never after posterior hypothalamic stimulation suggesting that there was little spread of current from one hypothalamic region to another (see Cross & Harris, 1952). Table 1 shows that, after stimulation of different hypothalamic regions, the proportion of females responding varied between the different groups, although there were no significant differences between body weights (range: 25·2±1·4 to 27·7±1·6 g; P>0·05) or ovarian weights (P>0·05). Anterior hypothalamic stimulation induced a higher proportion of females to ovulate than did mid-hypothalamic stimulation (χ² = 4·46; P<0·05), and the average number of corpora lutea present tended to be higher although the difference was not statistically significant (P>0·05). The number and distribution of stimulated follicles gave similar results. Stimulation of the posterior hypothalamic region did not induce ovulation and only one female had a stimulated follicle present.

Only six of the twelve females stimulated in the anterior hypothalamic region had Graafian follicles present, whereas ovaries from animals stimulated in the other two regions invariably had Graafian follicles when killed 24 hr later, the mean number being greatest in females stimulated in the posterior hypothalamus.
Control of ovulation in the vole

Median-eminence extract administration

Although no significant differences between body or ovarian weights in the experimental and control groups occurred, thirteen of the eighteen voles which received the median-eminence extract ovulated and all had stimulated follicles. No positive response occurred in the controls even though a full complement of Graafian follicles was present (see Table 2). All females which received the extract had Graafian follicles present at autopsy.

Table 1
EFFECT OF STIMULATION OF THE ANTERIOR, MID- AND POSTERIOR REGIONS OF THE HYPOTHALAMUS ON OVARIAN RESPONSE IN THE VOLE

<table>
<thead>
<tr>
<th>Stimulated region of hypothalamus</th>
<th>No. of voles</th>
<th>Ov. wt (mg) Mean ± S.E.</th>
<th>No. with corpora lutea</th>
<th>No. with stimulated follicles*</th>
<th>No. with Graafian follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>12</td>
<td>3.7 ± 0.3</td>
<td>(3.4 ± 0.7)</td>
<td>11 (4.7 ± 1.1)</td>
<td>6 (2.1 ± 0.7)</td>
</tr>
<tr>
<td>Mid</td>
<td>10</td>
<td>3.1 ± 0.3</td>
<td>(2.0 ± 1.0)</td>
<td>6 (2.7 ± 0.6)</td>
<td>10 (3.0 ± 0.5)</td>
</tr>
<tr>
<td>Posterior</td>
<td>7</td>
<td>4.1 ± 0.4</td>
<td>0</td>
<td>1† (4.9 ± 0.8)</td>
<td></td>
</tr>
</tbody>
</table>

Parentheses: indicates mean number (+ S.E.) present for individuals within each group.

* Stimulated follicles = luteinized follicles + follicles with strongly staining eosinophilic material + corpora lutea.
† Indicates total number present.

Table 2
EFFECT OF INTRAVENOUS INJECTION OF MEDIAN-EMINENCE EXTRACTS ON OVARIAN RESPONSE IN THE VOLE

<table>
<thead>
<tr>
<th>Treatment (injection)</th>
<th>No. of voles</th>
<th>Ov. wt (mg) Mean ± S.E.</th>
<th>No. with corpora lutea</th>
<th>No. with stimulated follicles</th>
<th>No. with Graafian follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median eminence extract</td>
<td>18</td>
<td>4.4 ± 0.3</td>
<td>13 (3.3 ± 0.5)</td>
<td>18 (4.7 ± 0.5)</td>
<td>18 (3.0 ± 0.4)</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>4</td>
<td>4.3 ± 0.6</td>
<td>0</td>
<td>0</td>
<td>4 (4.5 ± 0.6)</td>
</tr>
</tbody>
</table>

Presentation of data as in Table 1.

Luteinizing hormone administration (see Table 3)

There were no significant differences between body \(P>0.05\) or ovarian weights \(P>0.05\) but i.p. injections of 1.2, 2.5 and 5.0 \(\mu g\) of LH all gave a similar response. Ovulation occurred in all animals except for one vole given 1.2 \(\mu g\), two given 2.5 \(\mu g\), and two given 5 \(\mu g\). Only three of the six animals given 0.6 \(\mu g\) ovulated, however, and none did so if 0.3 \(\mu g\) or less was administered. Thus, the minimal amount of sheep LH required to induce ovulation following an i.p. injection is about 0.6 \(\mu g\). Graafian follicles were invariably
### Table 3

**EFFECT OF INTRAPERITONEAL AND INTRAVENOUS INJECTIONS OF LUTEINIZING HORMONE ON OVARIAN RESPONSE IN THE VOLE**

<table>
<thead>
<tr>
<th>Mode of injection</th>
<th>Dose (µg)</th>
<th>No. of voles</th>
<th>Body wt (g) Mean ± S.E.</th>
<th>Ov. wt (mg) Mean ± S.E.</th>
<th>No. with corpora lutea</th>
<th>No. with stimulated follicles</th>
<th>No. with Graafian follicles</th>
<th>Mean diameter of Graafian follicles (µ) (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal</td>
<td>1.2 to 5.0</td>
<td>24</td>
<td>26.4 ± 2.0</td>
<td>4.2 ± 0.5</td>
<td>19</td>
<td>21</td>
<td>24</td>
<td>449 ± 13</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>6</td>
<td>25.3 ± 2.3</td>
<td>3.9 ± 0.6</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>469 ± 14</td>
</tr>
<tr>
<td></td>
<td>0.3 to 0.07</td>
<td>10</td>
<td>26.4 ± 3.2</td>
<td>3.9 ± 0.9</td>
<td>3</td>
<td>0</td>
<td>10</td>
<td>506 ± 20</td>
</tr>
<tr>
<td>Intravenous</td>
<td>1.0 to 2.0</td>
<td>7</td>
<td>25.6 ± 2.0</td>
<td>5.1 ± 1.2</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>461 ± 11</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3</td>
<td>27.0 ± 2.1</td>
<td>4.5 ± 0.6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>493 ± 19</td>
</tr>
<tr>
<td></td>
<td>0.1 to 0.01</td>
<td>6</td>
<td>24.8 ± 2.1</td>
<td>4.0 ± 0.6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>506 ± 38</td>
</tr>
</tbody>
</table>

Presentation of data as in Table 1.
Control of ovulation in the vole

present even after injection of 5 \( \mu g \). The average size of the Graafian follicles present in females given the lower doses of LH was greater, thus suggesting that ripe follicles were present which would have ovulated had an appropriate stimulus been administered. Animals injected with 5 \( \mu g \) of LH did not have significantly more corpora lutea than those given lower doses.

Intravenous administration of LH had a similar effect to that of i.p. injections; more than 0·5 \( \mu g \) usually induced ovulation, whereas 0·1 \( \mu g \) or less did not.

DISCUSSION

These experiments provide further evidence that the vole is a reflex ovulator and indicate some of the neuro-anatomical and endocrinological pathways involved. The experiment involving electrical stimulation has shown that the anterior hypothalamus, or fibres passing through it, are implicated in controlling the release of LH from the anterior pituitary. Similar results have been obtained for the rat (Hillarp, 1949; Everett, 1965). Charlton, Naftolin & Worth (1970) have measured plasma LH after mating and after electrical stimulation of the anterior hypothalamus in the vole, demonstrating a marked rise after a few minutes. That neurohumours are involved in controlling the release of pituitary gonadotrophin is indicated by the experiment involving sheep median-eminence extract injections.

Twenty-four hours after mating, all Graafian follicles have usually disappeared (Breed & Clarke, 1970), but total absence in the present series of experiments was only found in animals after anterior hypothalamic stimulation. Thus, the administration of sheep median-eminence extract or sheep LH is not such an efficient ovulating stimulus, even if large doses are given. The route of injection of LH did not appear to affect the minimal amount required to induce ovulation.

ACKNOWLEDGMENTS

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REFERENCES