Effect of mating on plasma levels of LH and progesterone in montane voles (*Microtus montanus*)

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The effects of mating and other stimuli on ovulation have been reported for montane voles, *Microtus montanus* (Cross, 1972; Gray, Davis, Zerylnick & Dewsbury, 1974; Davis, Gray, Zerylnick & Dewsbury, 1974). These voles are induced ovulators, but no data are available on the precise hormonal changes involved. In the present study, plasma levels of LH and progesterone were measured in montane voles during various reproductive states.

All voles were laboratory bred and were 4–6 months of age. They were housed individually as adults in plastic tub cages (29 × 19 × 13 cm), with San-i-cel (Paxton Processing Co., Paxton, Illinois) and Nestlets (Ancare Corp., Manhasset, New York) provided for bedding. Purina Rabbit Chow (Ralston Purina Co., St Louis, Missouri) and water were available at all times, and fresh lettuce was provided once a week. Animals were maintained in a temperature-controlled room on a reversed 16 hr light/8 hr dark cycle (lights on 20.00 hours).

Concentrations of LH were measured by the ovine–ovine double-antibody radioimmunoassay described by Niswender, Midgley, Monroe & Reichert (1968) for the laboratory rat. Rat LH was employed as the reference standard (NIH-LH-RP1; potency = 0.03 NIH-LH-S1), and optimal precision occurred with 2–32 ng/tube. The intra-assay coefficient of variation was 10.4% (n = 6), and samples were run in duplicate. Progesterone was measured by the radioimmunoassay method of Thorneycroft & Stone (1973), except that samples were chromatographed on Sephadex LH-20 columns before assay. The least detectable dose of progesterone was 4 pg/tube, and plasma (50 µl) from ovariectomized and adrenalectomized rats contained 5.5 pg progesterone/tube. The intra-assay coefficient of variation was 8.6% (n = 4), and samples were run in duplicate.

In a preliminary study on the LH radioimmunoassay, dose–response inhibition curves for vole plasma and pituitary homogenates were compared with the inhibition curve for the rat LH standard. Nine males and nine females were decapitated 4 weeks after gonadectomy, and blood was collected into heparinized centrifuge tubes. The plasma was separated and frozen for subsequent assay. The pituitaries were homogenized in 1 ml saline and frozen. For radioimmunoassay, plasma samples were combined into male and female pools, and pituitary homogenates were diluted with 0.01 M phosphate-buffered saline–0.1% gelatin. Dose–response inhibition curves were plotted for the % of iodinated LH bound in the absence of competing unlabelled hormone against the log dose (ng/ml or µl/tube). The results showed that (1) an antigen in vole plasma and pituitaries, presumably LH, inhibited the binding of labelled LH by the antibody; and (2) in the area where binding was 20–80% of control, the curves for vole plasma and pituitary homogenates appeared parallel to the curve for rat LH. Statistical analysis of the slopes using analysis of variance revealed no significant differences between the curves. Although limited, these findings suggest that the assay is valid for measuring vole LH.

Plasma LH levels in males and females and progesterone levels in females were investigated in adults of proven fertility. The females were dioestrous, oestrous but unmated, or oestrous and mated. Dioestrous females were those housed individually and showing a vaginal smear containing 50% or more leucocytes on 2 consecutive days. Oestrous was elicited in females by placing them in one-half of a cage in which they were separated from a male by a hardware-cloth barrier (Gray et al., 1974). Vaginal smears containing 50% or more cornified cells for 2 consecutive days were considered

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indicative of oestrus. For mating, a female was placed in the cage of a sexually vigorous male 2 hr after the start of the dark period. Unreceptive females were excluded. Copulation was permitted for 1 hr following the first intromission. The female was then removed from the cage and decapitated within 1 min. Dioestrous and oestrous but unmated females were placed in a male's cage after removal of the male and decapitated 1 hr later.

Male voles, isolated from females for at least 2 weeks, were mated or unmated. For mating, the male's cage was placed on an observation shelf 2 hr after the start of the dark period and a receptive female was introduced. The male was decapitated 1 hr after the first intromission. The unmated males were decapitated 1 hr after being placed on the observation shelf.

**Table 1.** Mean (± S.E.M.) plasma levels of LH and progesterone in montane voles (nos in parentheses)

<table>
<thead>
<tr>
<th>Reproductive state</th>
<th>LH (ng RP-1/ml)</th>
<th>Progesterone (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioestrous</td>
<td>20.6 ± 5.9*</td>
<td>9.0 ± 0.9 (5)</td>
</tr>
<tr>
<td>Oestrous, unmated</td>
<td>23.1 ± 2.5*</td>
<td>14.0 ± 1.1 (5)</td>
</tr>
<tr>
<td>Oestrous, mated</td>
<td>896.1 ± 136.7</td>
<td>22.0 ± 1.5 (5)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmated</td>
<td>28.8 ± 3.6*</td>
<td>—</td>
</tr>
<tr>
<td>Mated</td>
<td>123.0 ± 40.5</td>
<td>—</td>
</tr>
</tbody>
</table>

* The concentration for at least one animal was < 2 ng/tube when 100 μl samples were run and was therefore considered as 20 ng/ml.

The results are shown in Table 1. Mating significantly increased plasma LH in males and females ($P < 0.01$). In the males, the increase was relatively small, and the LH concentration for 1 of the 6 mated animals was within the range of values for unmated males. Mating also significantly increased plasma progesterone levels in females ($P < 0.01$). Progesterone concentrations were significantly higher ($P < 0.05$) in oestrous unmated females than in dioestrous females.

The increases in plasma LH and progesterone concentrations of females 1 hr after mating are presumably involved in the ovulatory process. LH serves to trigger ovulation, and pronounced increases in circulating LH have been reported for field voles (*Microtus agrestis*: Charlton, Naftolin, Sood & Worth, 1975) and rabbits (Dufy-Barbe, Franchimont & Faure, 1973), which are induced ovulators. The increased progesterone levels, which have also been reported for rabbits after mating (Hilliard & Eaton, 1971; Fuchs & Beling, 1974), result from the stimulatory action of LH on the follicles although prolactin also may be involved. The function of preovulatory progestins may include: (1) stimulation of follicular rupture; (2) physiological actions on the oviduct, uterus and vagina; (3) enhancement of the LH surge through a positive feedback effect; (4) modulation of sexual receptivity (Goldman & Zarrow, 1973). The small increase in progesterone levels of oestrous but unmated females also may be involved in regulating receptivity. However, the source may be adrenal rather than ovarian, and it is possible that the increase represents an enhanced adrenal stress response to the testing procedures as a result of the presumably high oestrogen titres in oestrous females. The post-coital increase in plasma LH levels of male montane voles is similar to that found in male field voles (Charlot et al., 1975). Copulation-induced increases in gonadotrophins have been reported for several male mammals (Taleisnik, Caligaris & Astrada, 1966; Katongole, Naftolin & Short, 1971) although conflicting results exist (Convey, Bretschneider, Hafs & Oxender, 1971; Spies & Niswender, 1971; Lee, Jaffe & Midgley, 1974).

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References


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