Analysis of the LH surges induced by mating and LH-RH in the vole, *Microtus agrestis*

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Summary. Female voles mated with a male and experiencing a single ejaculatory series exhibited a rapid and marked elevation of plasma LH levels. The profile of the LH surge had a broad peak 20–40 min after mating. Pentobarbitone sodium administered immediately after mating did not affect the magnitude of the surge. The LH response to exogenous LH-RH was not affected by the state of receptivity of the female or by gonadectomy. There was no evidence of any priming effect of 25 ng LH-RH on the pituitary response to a second injection of LH-RH 20 or 60 min later. These results are consistent with the primary cause of the LH surge in the reflexly ovulating vole being a rapid secretion of LH-RH.

Introduction

The causes and characteristics of the preovulatory LH surge have been extensively studied in spontaneously ovulating mammals (e.g. women, monkeys, sheep, hamsters and rats: see Fink, 1979). Factors which contribute to the magnitude of the spontaneous LH surge include the increased secretion of LH-RH, a priming effect of LH-RH on the sensitivity of the pituitary to further LH-RH and a steroid-induced increase in responsiveness to LH-RH (Fink, 1979). Rather less attention has been paid to the causes and characteristics of the LH surge in animals that ovulate reflexly. Studies of the rabbit (Hilliard, Pang, Penardi & Sawyer, 1975; Carlson, Wong & Perrin, 1977) and vole (Charlton, Naftolin, Sood & Worth, 1975; Milligan & MacKinnon, 1976) and rats kept in constant light (Brown-Grant, Davidson & Greig, 1973) have shown that mating results in a very rapid rise in LH levels in the blood, presumably reflecting an increased secretion of LH-RH. It is not known whether changes in pituitary response to LH-RH due to steroid feedback and/or LH-RH priming are also involved and therefore the LH response of voles to mating and to LH-RH administration was examined.

Materials and General Methods

Animals. Laboratory-bred voles from the colony in the Department of Physiology, King's College, London, were maintained as described by Milligan (1974). Females were 2- and 5-month-old virgins exhibiting cornified smears. Males were breeding adults. For mating, an adult male was introduced into a cage containing a single female. Pairs that mated within 10 min of introduction were separated after the completion of the first ejaculatory series (Milligan, 1975b) by removing the male. In tests of receptivity of females, a small piece of adhesive plaster was placed over the vagina to prevent intromission before presenting the male for a 5-min period.

If no evidence of receptivity (lordosis response to mounting) was obtained in response to this first male, it was replaced by a second male for another 5-min period. Females that showed a lordosis response to either male were classed as receptive: those that failed to respond were classed as unreceptive. Previous observations (unpublished) have indicated that this assessment of receptivity corresponds closely to the willingness of unrestricted females to mate with a male within a 1-h period.

Injections and blood sampling. Animals were anaesthetized either briefly with ether or over a prolonged period with pentobarbitone sodium (Sagatal: May & Baker Ltd, Dagenham, England; diluted 1:5 in 0.154 M-NaCl; 0.1 ml (1.0 mg) administered/10 g body weight). In tests of the pituitary response to LH-RH, pentobarbitone sodium was administered 30 min before the LH-RH injection. LH-RH (the synthetic decapeptide; Hoechst U.K. Ltd) was dissolved in 0.154 M-NaCl. All injection volumes were 0.1 ml. Blood samples (normally 50–100 μ l) were obtained from the external jugular (using pentobarbitone sodium anaesthesia) or from the suborbital canthal sinus (ether anaesthesia). Plasma was stored at -20°C until hormone assay.

Radioimmunoassay. LH was measured by the assay described by Milligan & MacKinnon (1976) using NIH-LH-S19 as the standard. The inter- and intra-assay coefficients of variance were 10.0 and 19.1% respectively. The lower limit of sensitivity was 0.7 ng/ml.

Analysis of results. The mean increment over preinjection levels of LH in samples obtained at 10 and 20 min after LH-RH injection was measured. Analysis of variance and paired or unpaired Student's *t* tests were used as appropriate.

Detailed Methods and Results

Experiment 1: the LH surge induced by mating

Mated females were bled under light ether anaesthesia at 5, 10 and 15 min ($N = 6$; Group A); 10, 20 ($N = 17$) and 30 min ($N = 6$) (Group B); and 20, 30, 40 and 60 min ($N = 6$; Group C) after mating.

The plasma LH concentrations are summarized in Table 1. LH values in unmated females in our laboratory are routinely <5 ng/ml (Exps 3, 4 and 5; also Milligan, 1978) but mating resulted in much higher levels in all females at all times sampled. Student's paired *t* tests indicated that in Group A, levels at 10 min were significantly higher than at 5 min ($P < 0.01$), in Group B levels at 20 min were significantly higher than at 10 min ($P < 0.002$) and in Group C, levels at 30 min were higher than those at 20 min ($P < 0.01$) and those at 40 min were higher than those at 60 min ($P < 0.02$). Females from the different groups bled at similar times after mating showed no significant difference in LH concentrations. The combined data from these females and the females in Exp. 2, Group C (see below) suggest that the LH surge induced by mating has a rather broad profile with peak values occurring 20–40 min after mating.

Experiment 2: the effect of anaesthesia on the mating-induced LH surge

Mated females were anaesthetized with pentobarbitone sodium either immediately ($N = 9$; Group A) or 5 min ($N = 7$; Group B) after mating, or were anaesthetized with ether for each blood sampling procedure ($N = 8$; Group C). All animals were bled at 20, 60 and 100 min after mating.

There was no significant difference between the LH concentrations in the three groups of females at any of the sampling times (Table 1). Combining the results from all three groups, paired *t* tests revealed that LH values at 60 min *post coitum* were significantly lower than at 20 min ($P < 0.05$) and those at 100 min were significantly lower than those at 60 min ($P < 0.01$).

Table 1. The effect of mating and subsequent pentobarbitone sodium anaesthesia on mean $\pm$ s.e.m. plasma LH concentrations (ng/ml) in female voles

	No. of voles	Time (min) after mating							
		5	10	15	20	30	40	60	100
<i>Experiment 1 (mated only)</i>									
Group A	6	30.3 ± 5.78	42.8 ± 8.60	39.5 ± 8.98	—	—	—	—	—
Group B	17	—	37.1 ± 5.61	—	51.8 ± 8.18	45.5 ± 10.5 (6)	—	—	—
Group C	6	—	—	—	41.8 ± 16.8	66.7 ± 17.1	59.8 ± 14.7	46.5 ± 14.0	—
<i>Experiment 2</i>									
Pentobarbitone sodium immediately after mating (Group A)	9	—	—	—	52.3 ± 13.2	—	—	37.4 ± 4.3	25.8 ± 4.34
Pentobarbitone sodium 5 min after mating (Group B)	7	—	—	—	32.0 ± 9.02	—	—	24.7 ± 9.83	12.7 ± 4.02
Ether (Group C)	8	—	—	—	49.2 ± 12.1	—	—	33.1 ± 8.60	29.9 ± 6.50
Combined values of Exp. 1 and Group C of Exp. 2		30.3 ± 5.78	38.6 ± 4.73	39.5 ± 8.98	51.6 ± 6.13	57.1 ± 10.5	59.8 ± 14.7	38.8 ± 7.63	29.9 ± 6.50

Experiment 3: effect of state of receptivity and type of anaesthetic on the responsiveness to intravenous LH-RH

Six receptive and 8 unreceptive females were anaesthetized with pentobarbitone sodium. Ten additional females (5 receptive, 5 unreceptive) were anaesthetized with ether. Blood samples for hormone assay were taken immediately before and at 10 and 20 min after injection of 25 ng LH-RH.

There were no significant differences in the magnitude of the responses between any of the groups of females ($P < 0.05$) (Table 2).

Table 2. The effect of state of receptivity and type of anaesthetic on pituitary response to 25 ng LH-RH administered intravenously to female voles (Exp. 3)

	No. of voles	Mean $\pm$ s.e.m. plasma LH (ng/ml) after LH-RH			Mean $\pm$ s.e.m. increment over preinjection levels
		Preinjection	10 min	20 min	
Receptive (+ pentobarbitone sodium)	6	2.47 $\pm$ 0.45	36.8 $\pm$ 9.0	15.2 $\pm$ 4.3	23.6 $\pm$ 5.8
Unreceptive (+ pentobarbitone sodium)	8	1.55 $\pm$ 0.26	31.1 $\pm$ 2.8	8.75 $\pm$ 2.1	18.4 $\pm$ 2.2
Receptive (+ ether)	5	1.84 $\pm$ 0.38	28.5 $\pm$ 5.58	8.11 $\pm$ 1.11	16.5 $\pm$ 3.3
Unreceptive (+ ether)	5	2.18 $\pm$ 0.42	30.1 $\pm$ 10.1	15.0 $\pm$ 9.88	20.4 $\pm$ 14.0

Experiment 4: dose-response characteristics of LH-RH-induced LH secretion in intact and ovariectomized female voles

Intact females were anaesthetized with pentobarbitone sodium and injected with 5 (N = 5), 25 (N = 9), 100 (N = 8), 1000 (N = 6) or 10 000 (N = 6) ng LH-RH intravenously. Females ovariectomized 2 weeks previously were injected with 5 (N = 5), 25 (N = 5) or 100 (N = 7) ng LH-RH. Blood samples were taken from all females immediately before and at 10 and 20 min

after injection. Additional samples were taken at 60 min from those females given 1000 and 10 000 ng LH-RH. The intact females were examined for the presence of corpora lutea 4 days after injection.

In intact females, the magnitude and profile of the LH response was dependent on the amount of LH administered (Table 3). Increasing amounts of LH-RH released increasing amounts of LH. After injection of ≤ 100 ng LH-RH, the LH levels at 20 min were consistently lower than those at 10 min. After 1000 and 10 000 ng LH-RH, the LH surge was prolonged with the levels at 20 min being consistently higher than at 10 min, although by 60 min they had dropped below the 20-min values.

Table 3. The dose-response characteristics of LH-RH-induced LH secretion in intact and ovariectomized female voles (Exp. 4)

LH-RH (ng)	No. of voles	Mean $\pm$ s.e.m. plasma LH (ng/ml) after LH-RH				Mean $\pm$ s.e.m. increment at 10 and 20 min
		Preinjection	10 min	20 min	60 min	
<i>Intact females</i>						
5	5	6.60 $\pm$ 2.56	24.1 $\pm$ 6.0	14.9 $\pm$ 1.59	—	15.4 $\pm$ 5.10
25	9	2.71 $\pm$ 0.84	34.1 $\pm$ 3.27	27.6 $\pm$ 5.13	—	28.0 $\pm$ 3.22
100	8	3.40 $\pm$ 1.43	58.8 $\pm$ 6.38	32.7 $\pm$ 2.68	—	42.3 $\pm$ 3.42
1000	6	2.69 $\pm$ 0.26	52.6 $\pm$ 9.55	65.6 $\pm$ 14.0	35.3 $\pm$ 9.44	56.2 $\pm$ 11.35
10 000	6	5.18 $\pm$ 1.83	91.9 $\pm$ 27.1	121.0 $\pm$ 27.9	49.1 $\pm$ 27.9	89.1 $\pm$ 19.7
<i>Ovariectomized females</i>						
5	5	15.8 $\pm$ 4.84	48.1 $\pm$ 14.6	16.2 $\pm$ 3.34	—	16.3 $\pm$ 7.62
25	5	22.3 $\pm$ 8.57	87.1 $\pm$ 20.7	38.8 $\pm$ 11.5	—	40.6 $\pm$ 17.3
100	7	14.2 $\pm$ 3.06	115.7 $\pm$ 21.5	68.2 $\pm$ 12.7	—	77.8 $\pm$ 14.4

Ovariectomized females showed significantly higher preinjection LH levels (16.4 $\pm$ 2.92 ng/ml, $N = 17$) than did intact females (3.30 $\pm$ 0.58 ng/ml, $N = 34$) ($P < 0.01$). All but one ovariectomized female (treated with 5 ng LH-RH) exhibited an elevation in plasma LH after LH-RH administration. The magnitude of the response was not significantly different from that in intact females for 5 and 25 ng LH-RH but was significantly higher after 100 ng ($P < 0.025$).

The numbers of females ovulating in response to 5, 25, 100, 1000 and 10 000 ng LH-RH (including data from Exp. 3) were 0/5, 15/28, 7/8, 3/4 and 4/5 respectively and the respective mean numbers of corpora lutea per ovulating female were 3.4 $\pm$ 0.27, 3.86 $\pm$ 0.40, 4.0 $\pm$ 0.00, and 4.25 $\pm$ 0.25. All but 5 of the ovulating females developed corpora lutea that began to degenerate soon after their formation. Of those females given ≤ 100 ng LH-RH (i.e. showing an LH surge of short duration) the numbers ovulating after showing peak LH levels of ≤ 30 , 31–45 and ≥ 46 ng/ml were 5/15 (33%), 9/16 (56%) and 8/10 (80%) respectively. The mean number of corpora lutea in the ovulating females in these three categories was 3.20 $\pm$ 0.37, 3.38 $\pm$ 0.26, and 4.0 $\pm$ 0.33 respectively ($F = 1.75$, $P < 0.05$).

Experiment 5: effect of LH-RH priming on pituitary responsiveness to LH-RH

Two injections of 25 ng LH-RH were given to (a) intact females which had not been tested for receptivity, (b) females known to be receptive, and (c) adult males. The interval between the two LH-RH injections was 20 min (receptive females only) or 60 min (all treatments). All animals were anaesthetized with pentobarbitone sodium and blood samples were taken immediately before and at 10 and 20 min after each injection.

The results are summarized in Table 4. There were no significant differences between the responses to the first and second injections of LH-RH in any of the treatments involving female voles. In males, however, the response to the second injection was significantly smaller than the first ($P < 0.05$).

Table 4. The effect of LH-RH priming on pituitary responsiveness to LH-RH in female voles (Exp. 5)

	No. of voles	Mean $\pm$ s.e.m. plasma LH (ng/ml) after LH-RH						Mean $\pm$ s.e.m. increment over preinjection level	
		1st injection			2nd injection			1st injection	2nd injection
		Pre-injection	10 min	20 min	Pre-injection	10 min	20 min		
Mature females; no test of receptivity; 2 injections of 25 ng LH-RH 60 min apart	10	2.90 $\pm$ 0.92	42.3 $\pm$ 7.22	12.05 $\pm$ 1.96	1.74 $\pm$ 0.16	42.7 $\pm$ 9.58	35.5 $\pm$ 12.3	24.5 $\pm$ 4.48	37.5 $\pm$ 10.0
Receptive females; 2 injections of 25 ng LH-RH 20 min apart	7	4.78 $\pm$ 0.42	27.1 $\pm$ 5.23	16.7 $\pm$ 3.58		53.0 $\pm$ 16.3	24.2 $\pm$ 4.96	17.0 $\pm$ 4.0	21.9 $\pm$ 6.9
Receptive females; 2 injections of 25 ng LH-RH 60 min apart	10	2.39 $\pm$ 0.47	27.8 $\pm$ 6.59	18.0 $\pm$ 6.15	1.8 $\pm$ 0.30	30.2 $\pm$ 4.15	19.8 $\pm$ 4.5	20.5 $\pm$ 6.43	23.2 $\pm$ 3.94
Adult males; 2 injections of 25 ng LH-RH 60 min apart	6	2.75 $\pm$ 0.75	99.3 $\pm$ 27.3	54.0 $\pm$ 14.0	18.3 $\pm$ 3.9	83.3 $\pm$ 20.7	27.5 $\pm$ 5.5	74.6 $\pm$ 20.2	37.3 $\pm$ 10.5

Discussion

Mating in the vole consists of a number of ejaculatory series, each series being of one or more intromissions accompanied by pelvic thrusting (Milligan, 1975b). In the present study, it was found that females experiencing a single ejaculatory series exhibited a rapid rise in plasma LH with levels reaching a broad peak 20–40 min after mating (Exp. 1). Although it is not known to what extent the separate components of height and duration of the LH surge are important in determining the ovulatory response, in comparison with the LH surges induced by LH-RH and causing ovulation, there appeared to be a considerable safety factor involved in the mating-induced LH surge. The short-lived LH surge induced by ≤ 100 ng LH-RH caused ovulation in 50% of females showing peak (10 min) levels of 31–45 ng/ml but the LH response to mating was much longer and with mean peak levels of > 55 ng/ml. Similarly, in the spontaneously ovulating rat, Greig & Weisz (1973) reported that the minimal amount of LH required to induce full ovulation was equivalent to about 7% of the total preovulatory LH surge.

The extremely rapid initial rise in plasma LH levels is presumably primarily a result of the stimulation of LH-RH secretion. In the rat, pentobarbitone sodium inhibits LH-RH secretion (Greig & Weisz, 1973), but its administration to voles immediately after mating did not affect either the magnitude or time course of the LH surge (Exp. 2). Intravenous injection of 1000 ng LH-RH produced an LH response similar to that induced by mating (Exp. 4). These observations may indicate that the mating-induced LH-RH secretion is completed soon after mating. A lack of effect of pentobarbitone sodium on mating-induced LH secretion has been observed in rats kept in constant light (Brown-Grant *et al.*, 1973) and the rabbit (Sawyer, Markee & Everett, 1950). Despite the high levels of LH attained after administration of 1000 or 10 000 ng LH-RH in the vole, 5/7 of the ovulating females developed corpora lutea that started to degenerate soon after their formation. This confirms previous observations that the development of luteal function is not dependent on the magnitude of the LH surge, but rather on a separate neuroendocrine reflex that is normally activated by mating (Milligan, 1975a).

Although minimal amounts of mating are required to induce ovulation (Milligan, 1975a), under normal laboratory conditions of mating the female vole experiences multiple ejaculatory series within a short time period (Milligan, 1975b). It is not known whether separate LH secretory responses occur to each of these ejaculatory series or whether the response is all-or-none to the initial mating stimulus. Concannon, Hodgson & Lein (1980) found that the magnitude and duration of LH release in the cat were dependent on the number of copulations experienced. Charlton *et al.* (1975) reported that when a female vole was allowed to mate twice, with 90 min between the matings, separate LH surges were induced at each mating. The possibility of prolonged secretion of LH-RH after mating or of separate LH-RH secretory events associated with each mating attempt, raises the question of possible changes in pituitary sensitivity to LH-RH as a result of previous LH-RH exposure. In the rat, a self-priming effect of LH-RH on pituitary sensitivity is believed to be of major importance in determining the magnitude of the preovulatory LH surge (Aiyer, Chiappa & Fink, 1974). There was no evidence of any such priming effect in female voles 20 or 60 min after administration of 25 ng LH-RH i.v. (Exp. 5). In male voles, which also exhibit elevated LH levels following mating (Charlton *et al.*, 1975), the response to a second injection of LH-RH 60 min after the first was reduced.

The LH response to LH-RH was similar in receptive and non-receptive females (Exp. 3). Although the endocrine response basis of receptivity in the vole is unclear, the fact that ovariectomized females showed a similar (and even enhanced, as with 100 ng LH-RH) incremental response to LH-RH, suggests that gonadal steroids may have relatively little effect on acute pituitary responsiveness in the female. These results are in contrast to the situation in many spontaneous ovulators: in women, rhesus monkeys, sheep, hamsters and rats the pituitary responsiveness to LH-RH significantly increases both before and during the preovulatory LH surge (Fink, 1979) and oestradiol is essential for initiating and maintaining this increase in responsiveness in man and rat (Aiyer & Fink, 1974; Yen, Lasley, Wang, LeBlanc & Siler, 1975). Species differences are apparent in the effect of oestrogen and state of receptivity on pituitary responsiveness in induced ovulators. In the rabbit, the response to LH-RH was greater in females in spontaneous oestrus than in non-receptive does. Carlson & Perrin (1979) reported that the response was reduced by ovariectomy, but was restored in ovariectomized animals pretreated with oestrogen. In contrast, Dufy-Barbe, Franchimont, Dufy & Vincent (1975) reported that oestrogen priming inhibited the ability of LH-RH to stimulate LH release in ovariectomized does. In the ferret, Donovan & ter Haar (1977) observed that the gonadotrophin secretory response to LH-RH was greater in anoestrous and ovariectomized females and that treatment of ovariectomized females with oestrogen depressed responsiveness.

These observations indicate that the factors determining the characteristics of the preovulatory LH surge may differ not only between closely related species exhibiting reflex or spontaneous ovulation (i.e. vole and rat, respectively) but also between reflexly ovulating animals of different species.

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References

- Aiyer, M.S. & Fink, G. (1974) The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinising hormone releasing factor in the female rat. *J. Endocr.* **62**, 553–572.
- Aiyer, M.S., Chiappa, S.A. & Fink, G. (1974) A priming effect of luteinising hormone releasing factor on the anterior pituitary gland of the female rat. *J. Endocr.* **62**, 573–588.
- Brown-Grant, K., Davidson, J.M. & Greig, F. (1973)

- Induced ovulation in albino rats exposed to constant light. *J. Endocr.* **57**, 7–22.
- Carlson, J.C. & Perrin, D.G.** (1979) The effect of LH-RH administration on LH release in the female rabbit. *J. Reprod. Fert.* **56**, 175–180.
- Carlson, J.C., Wong, A.P. & Perrin, D.G.** (1977) The effect of prostaglandin and mating on release of LH in the female rabbit. *J. Reprod. Fert.* **51**, 87–92.
- Charlton, H.M., Naftolin, F., Sood, M.C. & Worth, R.W.** (1975) The effect of mating upon LH release in male and female voles of the species *Microtus agrestis*. *J. Reprod. Fert.* **42**, 167–170.
- Concannon, P., Hodgson, B. & Lein, D.** (1980) Reflex LH release in estrous cats following single and multiple copulations. *Biol. Reprod.* **23**, 111–117.
- Donovan, B.T. & ter Haar, M.B.** (1977) Effects of luteinising hormone releasing hormone on plasma follicle stimulating hormone and luteinising hormone levels in the ferret. *J. Endocr.* **73**, 37–52.
- Dufy-Barbe, L., Franchimont, P., Dufy, B. & Vincent, J.D.** (1975) Effect of ovariectomy and oestradiol benzoate treatment on LH-RH induced LH and FSH release in the female rabbit. *Horm. Res.* **6**, 372–379.
- Fink, G.** (1979) Neuroendocrine control of gonadotrophin secretion. *Br. med. Bull.* **35**, 155–160.
- Greig, F. & Weisz, J.** (1973) Preovulatory levels of luteinising hormone, the critical period and ovulation in rats. *J. Endocr.* **53**, 235–245.
- Hilliard, J., Pang, C.H., Penardi, R. & Sawyer, C.H.** (1975) Effect of coitus on serum levels of testosterone and LH in male and female rabbits. *Proc. Soc. exp. Biol. Med.* **149**, 1010–1014.
- Milligan, S.R.** (1974) Social environment and ovulation in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **41**, 35–47.
- Milligan, S.R.** (1975a) Mating, ovulation and corpus luteum function in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **46**, 97–100.
- Milligan, S.R.** (1975b) The copulatory behaviour of *Microtus agrestis*. *J. Mammal.* **56**, 220–224.
- Milligan, S.R.** (1978) The feedback of exogenous steroids on LH release and ovulation in the intact female vole (*Microtus agrestis*). *J. Reprod. Fert.* **54**, 309–311.
- Milligan, S.R. & MacKinnon, P.C.B.** (1976) Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*. *J. Reprod. Fert.* **47**, 111–113.
- Sawyer, C.H., Markee, J.E. & Everett, J.W.** (1950) Further experiments on blocking pituitary activation in the rabbit and rat. *J. exp. Zool.* **113**, 659–682.
- Yen, S.S.C., Lasley, B.L., Wang, C.F., LeBlanc, H. & Siler, T.M.** (1975) The operating characteristics of the hypothalamic-pituitary system during the menstrual cycle and observations of the biological action of somatostatin. *Recent Prog. Horm. Res.* **31**, 321–357.

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