The feedback of exogenous steroids on LH release and ovulation in the intact female vole (*Microtus agrestis*)

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Summary. Female voles were subjected to various regimens of subcutaneous injections of oestradiol-17 β , oestradiol benzoate and/or progesterone. Ovulation occurred in only a few of the mature females and in none of the immature animals. There was no indication of any increased LH levels in blood samples taken every 2 h for 50 h after 150 μ g oestradiol-17 β .

Introduction

The administration of oestrogen and/or progesterone can have a facilitatory effect on LH release and ovulation in many species and such feedback is now accepted as an essential part of the process leading to ovulation in spontaneously ovulating mammals (Brown-Grant, 1977). In animals which do not ovulate spontaneously ('induced' ovulators) the ovulatory LH surge is normally dependent upon mating (Harris & Naftolin, 1970), but the question of whether or not they also exhibit a positive feedback response to oestrogen and/or progesterone has received remarkably little attention. Scattered reports suggest that the administration of these steroids may result in ovulation in less than 50% of rabbits (Sawyer, Everett & Markee, 1950; Sawyer, 1959), and Kanematsu, Scaramuzzi, Hilliard & Sawyer (1974) observed a non-ovulatory peak of LH 8 h after the administration of 50 μ g oestradiol benzoate to oestrogen-primed does. There are no data for other induced ovulators. The present study was an investigation of the feedback effects of various steroids on ovulation and LH release in the vole, *Microtus agrestis*. This species is particularly suitable for such a study because it is an induced ovulator (Austin, 1957; Breed, 1967; Milligan, 1975a), with close phylogenetic relationship to the spontaneously ovulating rodents such as the rat and hamster in which the facilitatory effects of steroids are well established (Norman, Blake & Sawyer, 1973; Brown-Grant, 1977).

Materials and Methods

Voies from the breeding colony at King's College, London, were housed in groups of 3–5 and were maintained as described by Milligan (1974). In all except one treatment group in Exp. 1, in which the females were immature (5–6 weeks old), the animals were 2–5 months old and mature (as judged by the presence of cornified vaginal smears). The steroids (oestradiol-17 β , oestradiol benzoate, and progesterone: Sigma Chemical Co.) were dissolved in corn oil and administered subcutaneously in a volume of 0.1 ml. In Exp. 1, the treatment regimens were as indicated in Table 1. The females were killed or laparotomized 4 days after the final injection and the ovaries were examined macroscopically for the presence of corpora lutea (Milligan & MacKinnon, 1976). In Exp. 2, the effectiveness of other methods of inducing ovulation in comparable females was investigated. Females were injected subcutaneously with 10 μ g LH (NIH-LH-S19) in 0.1 ml saline (0.154 M-NaCl) or intravenously with 100 ng LH-RH (the synthetic decapeptide: Hoechst Pharmaceuticals) in 0.1 ml saline, or were allowed to mate with a male until one ejaculation had been completed (Milligan, 1975b). In Exp. 3, the LH secretory response of females to a subcutaneous injection of 150 μ g oestradiol-17 β given at 09:00 h was investigated. The relatively small size of *M. agrestis* (adult weight 20–40 g) prevented long-term

serial blood sampling; each female was therefore bled 3 or 4 times (with 2-h intervals between successive bleedings) and different groups of females (5/group) were used to extend the sampling period to 50 h after the steroid injection. Blood samples (0.1 ml) were taken from the suborbital canthal sinus under light ether anaesthesia and LH was measured by the radioimmunoassay described by Milligan & MacKinnon (1976) and using NIH-LH-S19 as the standard. The intra- and inter-assay coefficients of variance were 10.7 and 20.0% respectively. The lower limit of sensitivity of the assay was 0.7 ng/ml.

Results

The results of Exps 1 and 2 are summarized in Table 1, as are the data on the ovulatory responses of the females in Exp. 3. While LH or LH-RH treatment induced ovulation in 60–70% of the females and copulation caused ovulation in all the females that mated (Exp. 2), none of the steroid treatments resulted in more than a small proportion of the animals ovulating. No corpora lutea were found in any of the 20 control females treated with oil alone, or in any of the immature females treated with oestradiol-17 β .

The mean ($\pm$ s.e.m.) plasma LH concentration in 10 untreated mature females in this laboratory colony was 2.2 ± 0.22 ng/ml. In Exp. 3, no marked elevation in the plasma concentration of LH was apparent within 50 h of the oestradiol-17 β injection. Only 2 females possessed corpora lutea when examined 4 days after the injection, but neither showed elevated levels of LH (sampled between 2 and 6 h and between 20 and 24 h after injection, respectively). The highest value recorded for any female was 14.0 ng/ml, but this was not accompanied by ovulation. All other samples showed levels below 9.0 ng/ml and 93.5% of all samples contained <5.0 ng LH/ml. The mean ($\pm$ s.e.m.) plasma LH concentration for all samples was 3.04 ± 0.21 ng/ml ($n = 88$).

Table 1. The effect of treatment with oestradiol-17 β , oestradiol benzoate, progesterone, LH, LH-RH or mating on ovulation in voles

Treatment	No. of animals		No. of CL/ovulating vole (mean $\pm$ s.e.m.)
	Treated	Ovulating (%)	

<i>Experiment 1</i>			
None (oil only)	20	0 (0)	—
Oestradiol-17 β			
0.5 μ g	4	0 (0)	—
5.0 μ g	4	0 (0)	—
50 μ g	4	1 (25)	3
50 μ g (immature females)	8	0 (0)	—
150 μ g (caged in groups)	7	1 (14.3)	4
150 μ g (caged separately)	7	2 (28.6)	6, 3
150 μ g (females in Exp. 3)	40	2 (5)	5, 4
Oestradiol benzoate			
50 μ g	12	2 (16.7)	4, 4
50 μ g + progesterone (1 mg)	11	2 (18.1)	2, 4
20 μ g + progesterone (1 mg) 72 h later	12	2 (16.7)	2, 4
20 μ g + oestradiol benzoate (50 μ g) 72 h later	12	2 (16.7)	6
Progesterone (1 mg)	12	1 (8.3)	6

<i>Experiment 2</i>			
LH (10 μ g)	10	6 (60)	5.3 $\pm$ 0.81
LH-RH (100 ng)	13	9 (70)	4.1 $\pm$ 0.65
Mating	10*	10 (100)	4.7 $\pm$ 0.50

* Of 14 females tested.

Discussion

There was no evidence of a well developed positive-feedback response to exogenous oestradiol and/or progesterone in female voles (Exp. 1), despite the fact that the regimens of steroid administration were similar or identical to these that are highly effective in causing LH release and ovulation in rats (Brown-Grant, 1977), and the fact that most of the doses of oestradiol-17 β and oestradiol benzoate were greatly above those required to cause other oestrogenic responses. Vaginal perforation and cornification are reliably induced by 1.0 μ g oestradiol and receptivity by 10.0 μ g oestradiol benzoate or -17 β (unpublished observation).

The cause of the few ovulations that did occur is uncertain. While they may have been the result of a direct facilitatory feedback of steroids on LH release, they may also have been due to an indirect effect, such as increasing responsiveness to other ovulatory stimuli (Sawyer *et al.*, 1950; Milligan, 1974). Direct social interaction (e.g. mounting behaviour) is unlikely to have caused all these ovulations, however, because corpora lutea were still found in 2 of 7 females caged separately and given 150 μ g oestradiol-17 β (see Table 1). The possibility that the steroid treatments resulted in a general facilitation of LH secretion, but that this was only of sufficient magnitude to induce ovulation in a few females, seems unlikely because no major surges of LH were observed during the 50 h after oestradiol-17 β treatment and few values exceeded 5 ng LH/ml. Such levels are similar to those seen in mature, untreated females and are considerably below those which occur during the ovulatory surge following mating (peak value after mating 66.7 ± 17 ng/ml; $N = 6$; see also Charlton, Naftolin, Sood & Worth, 1975). After an injection of 5–100 ng LH-RH, which produces a more transient LH surge than mating, peak levels of LH of more than 30 ng/ml are required to induce ovulation in 50% of animals (unpublished observations).

The apparent absence of a marked facilitatory effect of steroids on LH release in the vole is in marked contrast to the situation in spontaneously ovulating rodents (Norman *et al.*, 1973; Brown-Grant, 1977). Whether this reflects an actual absence of the sexually differentiated gonadotrophin response characteristic of spontaneous ovulators, or whether the potential response in the vole is merely blocked by other endogenous hormones (e.g. progesterone: Blake, 1977) has yet to be determined.

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