The effect of LH-RH administration on LH release in the female rabbit

J. C. Carlson and D. G. Perrin

Biology Department, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Summary. Intracarotid infusion of LH-RH to female rabbits stimulated a significant increase in plasma LH concentration in the jugular vein. This response varied with the reproductive state of the animal, with a greater release occurring in oestrous (spontaneous or oestrogen-induced) and non-receptive does than in pseudopregnant or ovariectomized animals. If ovariectomized rabbits were pretreated with oestrogen, the pituitary response to LH-RH was restored. These findings suggest that there is little change in pituitary sensitivity to LH-RH infusion between oestrous and non-receptive rabbits, although pseudopregnancy (high physiological levels of progesterone) or ovariectomy inhibit its ability to respond to a releasing-hormone stimulus.

Introduction

In the spontaneous ovulator, basal levels of LH are released throughout the reproductive cycle except for a large surge preceding ovulation. LH-releasing hormone (LH-RH) from the hypothalamus appears to control the secretion of LH from the pituitary gland. For example, experimental administration of this hormone causes LH secretion in a number of species and pituitary stalk plasma LH-RH levels increase at the time of the preovulatory LH surge in the rat (Sarkar, Chiappa, Fink & Sherwood, 1976). The stage of the oestrous cycle has been shown to influence the pituitary response to LH-RH, with the greatest increase occurring on the afternoon of pro-oestrus (Cooper, Fawcett & McCann, 1973; Gordon & Reichlin, 1974).

The rabbit is a reflex ovulator requiring the mating stimulus to trigger the LH surge. Although recent studies in this species indicate that LH-RH administration can stimulate an ovulatory release of LH (Amoss, Blackwell & Guillemin, 1972; Kanematsu, Scaramuzzi, Hilliard & Sawyer, 1974; Dufy-Barbe, Franchimont, Dufy & Vincent, 1975), the effects that ovarian hormones have upon this response are unclear. The ability of LH-RH administration to cause LH release in the female rabbit during various reproductive conditions was therefore examined.

Materials and Methods

Animals and experiments

The 54 mature (3–5 kg) New Zealand White does were housed individually in a room with 14 h light/24 h and each animal had free access to food and water. All experiments were carried out between June and December.

On the day of treatment, each animal was anaesthetized with approximately 1 mg pento-barbitone sodium (Abbott Laboratories, Ltd, Montreal) and an indwelling cannula was inserted into the jugular vein for blood collection and into the carotid artery for administration of LH-RH. LH-RH was dissolved in 0.9% (w/v) NaCl solution and diluted so that a constant volume of 7.5 ml/30 min was infused.

The rabbits were divided into 10 groups of 4–5 animals each. The does in Groups 1–6 received a 30-min control infusion (7.5 ml) of 0.9% (w/v) NaCl followed by three 30-min infusions of LH-RH at 100, 500 and 2500 ng respectively. Groups 7–10 also received a similar 30-min control infusion followed by a constant infusion of LH-RH at 1 µg/h for 2 h. Serial blood samples (1.5 ml each) were withdrawn, combined with heparin (100 units) and centrifuged. The plasma samples were stored at –20°C until assay. The animals were non-receptive does (Groups 1 and 7), spontaneous oestrous does (Groups 2 and 8), pseudopregnant does (Groups 3 and 9), oestradiol benzoate-primed oestrous does (Groups 4 and 10), ovariectomized does (Group 5) and ovariectomized does treated with oestradiol benzoate (Group 6). On the day of treatment with LH-RH the does in Groups 1, 2, 4, 7, 8 and 10 were assigned to their respective groups after examination for oestrous behaviour with a vasectomized buck. Mating was prevented in oestrous animals. The rabbits in Groups 3 and 9 were mated to a vasectomized male on Day 0 and treated on Days 9, 10 or 11 of pseudopregnancy. The does in Groups 4, 6 and 10 were pretreated with subcutaneous injections of 50 µg oestradiol benzoate (Sigma Chemical Co., St Louis)/day for 2 consecutive days and LH-RH on the 3rd day. In Groups 5 and 6, bilateral ovariectomy was performed at least 2 months before experimentation.

LH assay

Plasma LH concentrations were determined with a heterologous radioimmunoassay similar to that described previously (Carlson, Wong & Perrin, 1977). The major difference was that the antiserum to rabbit LH (6FGP) was raised in a guinea-pig. This antiserum cross-reacted less than 1% with rabbit FSH and GH, 5.7% with rabbit TSH and 100% with ovine LH. The assay sensitivity was 250 pg/ml plasma. Serial dilutions of rabbit plasma were parallel to the rabbit LH standard, EX 130 GP. Each unknown sample was assayed in duplicate using 10–50 µl plasma. The between-assay coefficient of variation was 13.9% and the within-assay coefficient of variation was 3.7%.

Results

Administration of LH-RH in increasing doses stimulated release of LH in each of the 6 groups examined (Text-fig. 1). The increase in plasma LH during the 100 ng infusion was significantly ($P < 0.05$, paired t test) greater than the values recorded during the preceding control period in all groups except for the pseudopregnant animals of Group 3 (Table 1). Although plasma LH

Table 1. Jugular plasma LH concentrations (ng/ml) during intracarotid infusion of LH-RH in the rabbit

Group	Reproductive state	No. of rabbits	Dose of LH-RH (ng/30 min)			
			Saline	100	500	2500
1	Non-receptive	5	2.6 ± 0.13	4.9 ± 0.70*	22.9 ± 3.64**	56.6 ± 5.14**
2	Spontaneous oestrous	5	2.4 ± 0.18	11.1 ± 2.90*	46.4 ± 6.19**	67.3 ± 2.45**
3	Pseudopregnant	5	2.0 ± 0.13	3.8 ± 0.44	4.6 ± 0.48*	20.0 ± 2.54**
4	Oestradiol benzoate-primed oestrous	5	2.4 ± 0.25	23.2 ± 2.34**	38.6 ± 3.11**	63.0 ± 3.44**
5	Ovariectomized	5	12.2 ± 0.71	23.4 ± 0.52**	25.8 ± 1.18**	33.3 ± 2.01**
6	Ovariectomized and oestradiol benzoate-primed	5	1.8 ± 0.43	15.9 ± 2.41**	27.8 ± 1.94**	69.7 ± 9.37**

Each value represents the mean (±s.e.m.) of all samples collected at each dose for that group.

Values significantly different from that during saline infusion: * $P < 0.05$, ** $P < 0.01$ (paired t test).

levels increased more rapidly in the spontaneously oestrous rabbits (Group 2) than in the non-receptive does (Group 1), the overall response was similar in both groups and the maximal concentrations of LH were not significantly different. In contrast, the highest plasma LH concentration attained in the pseudopregnant rabbits was significantly ($P < 0.01$) less, being about one-third of that observed in Groups 1 and 2.

In Groups 4–6 there was an immediate response to treatment (Text-fig. 1), as detectable increases in plasma LH concentration appeared within 10 min. In the oestrogen-primed oestrous rabbits of Group 4, plasma LH levels rose more quickly than in the spontaneously oestrous

Text-fig. 1. The effect (mean $\pm$ s.e.m.) of stepwise intracarotid infusion of LH-RH (100, 500 and 2500 ng/30 min) on jugular plasma LH concentrations in non-receptive (Group 1), oestrous (Group 2), pseudopregnant (Group 3), oestradiol benzoate-primed (Group 4), ovariectomized (Group 5) and oestradiol benzoate-primed ovariectomized (Group 6) rabbits (5/group).

rabbits of Group 2, but the maximal concentrations were not statistically different. Before treatment jugular LH levels in the ovariectomized does (Group 5) were approximately 5-fold higher than in intact animals. With the start of LH-RH infusion, plasma concentrations of LH doubled, but further significant elevations at higher doses did not occur. Plasma levels of LH returned to basal values of 2–3 ng/ml following pretreatment with oestradiol benzoate in Group 6. Initially, the release of LH followed a pattern similar to that of the ovariectomized does of Group 5, but at the highest infusion rate, there was a second increase in LH release which was not apparent in Group 5.

To check whether there was any effect of the 2-h infusion period on jugular LH concentration, an additional 1 or 2 animals representative of each of the above groups were infused with saline for 2 h. No change in plasma LH concentrations appeared.

Infusion of LH-RH at a constant dose of 1 µg/h for 2 h also stimulated a significant increase in plasma LH concentration in Groups 7–10 (Table 2). The maximal levels of LH attained were not significantly different in the 2 groups of oestrous does (Groups 8 and 10). The increase was significantly lower in Group 7, the non-receptive animals ($P < 0.05$) and in Group 9, the pseudo-pregnant does ($P < 0.01$). As with the multiple-dose infusion, plasma LH concentration rose fastest in the oestradiol benzoate-induced oestrous animals.

Table 2. Jugular LH concentrations (ng/ml) during intracarotid infusion to does of LH-RH at 1 µg/h for 2 h

Group	Reproductive state	No. of rabbits	Time (min)							
			0	20	40	60	80	100	120	140
7	Non-receptive	4	1.5 ± 0.5	2.3 ± 0.5	3.8 ± 0.6	10.1 ± 3.0	18.4 ± 6.4	23.8 ± 8.0	30.1 ± 8.1	25.5 ± 6.9
8	Spontaneous oestrous	4	1.9 ± 0.1	6.3 ± 2.5	18.7 ± 4.4	44.2 ± 10.5	58.7 ± 1.9	57.6 ± 7.9	49.9 ± 2.5	32.0 ± 5.5
9	Pseudopregnant	4	2.0 ± 0.3	3.7 ± 0.1	3.4 ± 0.3	3.6 ± 0.1	4.1 ± 0.1	6.1 ± 0.2	9.5 ± 1.1	3.3 ± 0.4
10	Oestradiol benzoate-primed oestrous	4	1.3 ± 0.1	20.8 ± 4.6	28.3 ± 3.5	27.1 ± 3.2	31.8 ± 6.8	32.4 ± 7.3	34.3 ± 8.2	23.6 ± 5.5

Each value represents the mean (±s.e.m.) of all samples collected in that group at each time.

The increase in LH concentration was significantly greater in Groups 8 and 10 than in Group 7 ($P < 0.05$) and Group 9 ($P < 0.01$).

Within 4–5 days of LH-RH administration, the ovaries of animals in Groups 1, 2, 4, and 7–10 were examined for corpora lutea to check whether ovulation had occurred. Corpora lutea of normal numbers and appearance (6–10 per animal) were found in Groups 2 and 4. Fewer CL (2–5 per animal) were observed in Groups 8 and 10 and there were none in Groups 1, 7 and 9.

Discussion

Administration of LH-RH in the female rabbit readily stimulated LH release as reported previously by Kanematsu *et al.* (1974) and Dufy-Barbe *et al.* (1975). The amount of this gonadotrophin released during infusion resulted in peak concentrations similar to that caused by mating (Carlson *et al.*, 1977) and was sufficient to induce ovulation. In addition, our observations indicate that oestrogen pretreatment sensitizes the anterior pituitary, causing LH to be released more quickly in intact animals or in greater amounts in ovariectomized does during releasing-hormone infusion. Our findings in ovariectomized rabbits differ from those of Dufy-Barbe *et al.* (1975) who reported that oestrogen priming inhibited the ability of LH-RH to stimulate LH release in ovariectomized does. Comparison with their results is difficult because of different means of administration (intravenous injection versus intracarotid infusion). Although our approach by infusion may have more closely simulated the pattern of endogenous LH-RH

secretion before ovulation in this species (Tsou *et al.*, 1977), it is also possible that the initial dose of releasing hormone (100 ng) produced a priming effect that resulted in a much larger LH release during the second hour of infusion. A priming effect, in which subsequent injections of LH-RH evoke a greater release of LH, has been demonstrated previously in the rat (Fink, Chiappa & Aiyer, 1976) and ewe (Crighton & Foster, 1977). To examine this possibility in the rabbit, we infused LH-RH at a constant rate for 2 h in Groups 7–10. In the oestrous does (spontaneous and oestrogen-primed) the LH release during infusion was rapid, suggesting that there was little if any priming effect. Although the delayed release of LH in non-receptive and pseudopregnant does may indicate a priming effect, it may also be caused by a gradual increase in LH-RH concentration in the plasma as the infusion progressed.

Experiments with LH-RH in the rat indicate that there is a marked change in pituitary sensitivity to releasing hormone during the oestrous cycle with the greatest response occurring at pro-oestrus (Cooper *et al.*, 1973; Gordon & Reichlin, 1974; Ferland, Borgeat, Labrie, Bernard & DeLean, 1975; Legan & Karsch, 1975). This change appears to be related to the effects of oestrogen on the anterior pituitary, because circulating levels of endogenous oestradiol-17 β increase at this time (Butcher, Collins & Fugo, 1974) and pretreatment with oestradiol benzoate raises the amount of LH released by LH-RH treatment in dioestrous (Arimura & Schally, 1971) and ovariectomized (Libertun, Cooper, Fawcett & McCann, 1974) rats. Less is known about plasma levels of oestrogen in the unmated rabbit although Perrin (1976) has shown that in the days preceding spontaneous oestrus in untreated does, plasma oestradiol-17 β levels are low except for occasional small peaks. These observations, together with our findings in the present study, suggest that a minimal level of circulating oestrogen is necessary to maintain pituitary sensitivity so that sufficient amounts of LH are released for ovulation after mating.

Goodman & Neill (1976) have reported that oestradiol benzoate pretreatment in ovariectomized rabbits does not restore the ability of the hypothalamic–pituitary complex to release a normal ovulatory LH surge after mating. The reasons for this discrepancy with our results for oestrogen-primed ovariectomized rabbits treated with LH-RH (Group 6) are uncertain. It is possible that the positive effects of oestrogen pretreatment may occur mainly at the pituitary gland and not at the hypothalamus under these experimental conditions. Alternatively, there may be a second ovarian hormone necessary for preparing the hypothalamus to release LH-RH at mating. Despite this uncertainty, however, it appears that one important site in the feedback role of oestrogen is the pituitary gland.

Oestrogen and progesterone exhibited negative feedback effects in this study. Oestradiol benzoate treatment to ovariectomized rabbits (Group 6) restored basal levels of LH in the jugular plasma, as observed by Dufy-Barbe *et al.* (1975). Oestrogen therefore appears responsible for limiting LH secretion in the rabbit except after mating. In addition, the high progesterone levels found during pseudopregnancy (Carlson & Gole, 1978) inhibit stimulation of pituitary secretion by LH-RH. Hilliard, Schally & Sawyer (1971) have reported that progesterone blocked ovulation in LH-RH-treated rabbits. A major site for the negative feedback influence of progesterone appears to be the pituitary, since LH-RH administration bypasses the hypothalamus. However, a hypothalamic site may be considered if continuous release of endogenous LH-RH is necessary for maintaining pituitary capacity to secrete LH. Like Hilliard *et al.* (1971), we have found that higher doses of LH-RH can partly overcome the negative feedback influence of progesterone; plasma LH levels rose significantly during infusion of LH-RH at the highest dose in pseudopregnant rabbits.

We thank Dr R. J. Scaramuzzi, Blacktown, New South Wales, for the antiserum, Dr L. E. Reichert, Jr, Emory University, Atlanta, Georgia, for the purified ovine LH (LER-1056-C2), Dr H. Papkoff, University of California, San Francisco, for the purified rabbit LH standard (EX 130 GB) and the NIAMDD, Bethesda, Maryland for the LH-RH.

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Received 22 August 1978