Differential release of LH and FSH in cyclic mares in response to synthetic Gn-RH

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Summary. Mares at different stages of the oestrous cycle were given a single intravenous injection of 0.5 mg synthetic Gn-RH.

The mean area of the induced LH peak was significantly less at mid-cycle (Day 10–11) than at any other time. The mean height of the LH peak above preinjection concentration was greater at late oestrus and early cycle (Day 5–6) than at mid-cycle and early oestrus. There were no significant differences in mean FSH responses. The LH:FSH ratio for both height and area of induced peaks was significantly less at mid-cycle than at other times of injection. These results suggest that one releasing hormone could cause the release of both FSH and LH in the normal cyclic mare.

Introduction

The pattern of release of gonadotrophins during the oestrous cycle of the mare has been shown to be different from that of other large domestic species. Serum luteinizing hormone (LH) concentrations in the mare do not show a large preovulatory peak of only a few hours duration but increase gradually during the first 4–5 days of oestrus, and reach a peak 1 or 2 days after ovulation (Pattison, Chen & King, 1972; Whitmore, Wentworth & Ginther, 1973; Noden, Oxender & Hafs, 1974; Evans & Irvine, 1975; Geschwind, Dewey, Hughes, Evans & Stabenfeldt, 1975; Nett, Holtan & Estergreen, 1975; Nett, Pickett, Seidel & Voss, 1976). There are two distinct surges of FSH in the serum of mares, one in late oestrus or early dioestrus and one in mid- to late-dioestrus (Evans & Irvine, 1975). The former is coincident with increased LH release but the latter occurs at a time when plasma LH concentration is basal. It is possible that this differential release is due to separate FSH and LH releasing hormones. However, preliminary experiments in acyclic mares suggest that one gonadotrophin-releasing hormone can induce differential FSH and LH release (Evans & Irvine, 1976a). This would be possible if, for example, pituitary responsiveness to the releasing hormone changes separately for LH and FSH in different reproductive situations.

The pituitary responsiveness of mares to synthetic gonadotrophin-releasing hormone (Gn-RH) was therefore tested at various stages of the oestrous cycle. This material had previously been shown to release LH and FSH in seasonally anoestrous mares (Evans & Irvine, 1976b, 1977).

Materials and Methods

Experiments. Five Standardbred mares grazing on pasture at Lincoln College Research Farm were bled daily (in the late morning or early afternoon) by jugular venepuncture throughout the

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experiment (October 1977 to April 1978, a period which encompassed the physiological breeding season). Occurrence of oestrus and ovulation was determined by regular teasing by a stallion, palpation of the ovaries *per rectum*, and serum progesterone concentrations. These mares were used for more than one treatment group but at least one complete normal oestrous cycle was allowed to elapse between each treatment. Some mares developed a persistent corpus luteum and lysis was induced by a prostaglandin injection. In these mares Gn-RH treatment was not given until the following oestrous cycle to avoid any possible effect of the prostaglandin injection on the results obtained. In addition 4 mares from the Nevele R Stud were used once in the experiment at a known stage of oestrus.

Groups of mares were given an intravenous injection of 0.5 mg Gn-RH (Hoechst Pharmaceuticals Ltd.) at 09:00 h during early oestrus, late oestrus, Day 5–6 after ovulation (early cycle) or Day 10–11 after ovulation (mid-cycle). The stage of the cycle at which Gn-RH was given was determined by comparing the daily serum FSH, LH and progesterone changes before injection with the levels in the normal cycle. Each mare given Gn-RH was sampled by jugular venepuncture at –0.5, –0.25, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h in relation to the injection (Time 0). The blood samples were allowed to clot overnight at 4°C and the serum was then collected and stored at –20°C until assay.

Hormone assays. Serum FSH and LH concentrations were determined in all samples. FSH was measured by a previously described radioimmunoassay method (Evans & Irvine, 1976b) using anti-human FSH antiserum with labelled human FSH. The Nuti equine FSH used as standard had a potency of 90 times that of NIH-FSH-S1 when determined by the Steelman–Pohley assay (Braselton & McShan, 1970). At a final dilution of 1:31 500, the antibody specifically bound 16–22% of the ¹²⁵I-labelled hFSH; non-specific binding was $1.67 \pm 0.2\%$. The intra- and inter-assay coefficients of variation were 12.3 and 12.8% respectively.

Serum LH concentrations were measured using a modification of the method of Noden *et al.* (1974). At a final dilution of 1:213 000 the anti-ovine LH antiserum specifically bound 15–35% of the ¹²⁵I-labelled oLH; non-specific binding was $3.18 \pm 1.63\%$. The Nuti equine LH used as standard had 5.3 times the activity of NIH-LH-S1 when assayed by the ovarian ascorbic acid depletion assay and 33.6 times the activity when determined by the ventral prostate weight assay (Braselton & McShan, 1970). The intra- and inter-assay coefficients of variation were 11.6 and 15.6% respectively. The progesterone concentration was measured in a proportion of the daily samples so that the serum concentration was known every 3rd day in mid-cycle, every 2nd day before, during and after oestrus, and daily around the time of Gn-RH treatment. The method used was that of Berwyn-Jones & Irvine (1974).

The LH and FSH responses to Gn-RH injection were assessed in terms of height and area of the hormone peaks above the preinjection baseline. The area was measured in arbitrary units on graph paper. The duration of the response was not used since this was sometimes greater than the time of sampling and at other times was difficult to assess accurately. In animals in which the concentration had not returned to baseline by the end of the sampling period (2 occasions) the area was also underestimated, but this underestimate would have been much smaller as a percentage of the total area than would be the case for the underestimate in response duration.

The actual values for LH and FSH cannot be directly compared, but the LH:FSH ratio for both peak height and area gives a comparison of the relative changes in LH and FSH responsiveness to Gn-RH during the cycle. The means of the different groups were compared by Student's *t* test.

Results and Discussion

The FSH and LH concentrations in 5 normal oestrous cycles in 3 mares are shown in Text-fig. 1(a). Two surges of FSH occurred in each cycle, one around the time of oestrus and one at mid-cycle. The timing of both these was variable between and within animals and hence the mean

Text-fig. 1. Mean $\pm$ s.e.m. changes in (a) serum LH (■) and FSH (□) concentrations in 5 normal oestrous cycles from 3 different mares (oestrus was approximately from 5 days before ovulation until the day of ovulation, Day 0) and (b) areas of LH (solid columns) and FSH (open columns) peaks induced by intravenous Gn-RH injection at various stages of the cycle. The mean ($\pm$ s.e.m.) LH:FSH ratio is shown above each pair of columns.

serum FSH graph shows less distinct surges than individual animals. This also accounts for the large standard error in the FSH values. This pattern of FSH concentrations was similar to that found previously, although the lowest concentrations of the cycle occurred following the early cycle surge rather than in early oestrus as reported by Evans & Irvine (1975). Serum LH always showed an increase during oestrus which lasted for several days, reached a peak in late oestrus, and did not start to decline until 1 or 2 days after ovulation. This pattern is similar to that reported by Noden *et al.* (1975) who used a comparable assay system.

There was no significant difference in the FSH responses at various times of the cycle to Gn-RH injection (Table 1, Text-fig. 1b). However, all the higher responses were in the early and mid-cycle groups and there was a very low FSH response in two of the animals injected in early oestrus. Thus if anything the FSH response was greater at mid-cycle than it was at oestrus.

The LH response to synthetic Gn-RH in terms of area of the induced peak was significantly less at mid-cycle than at all the other times tested (Text-fig. 1b). No other times of the cycle had a significantly different response in terms of area, but the height of the induced LH peak was significantly greater when the injection was given at late oestrus than when it was given in early oestrus (Table 1), despite the fact that the start of the natural increase in LH concentration was imminent at the latter time. In other species the responsiveness of the pituitary gland to a single injection of synthetic or purified Gn-RH is generally highest at times when the preovulatory LH release is imminent, for example in the rat (Cooper, Fawcett & McCann, 1973), golden hamster (Arimura, Debeljuk & Schally, 1972), rhesus monkey (Krey, Yamaji, Butler & Knobil, 1972) and sheep (Reeves, Arimura & Schally, 1971; Foster & Crighton, 1976a, b). In the present experiment the largest LH response to Gn-RH was, however, at late oestrus which coincides with the time when the serum LH concentration is at its highest. Since the response is measured in terms of size of peak above preinjection values this shows that although the LH concentration is high when the injection is given at late oestrus it is capable of being stimulated further. The LH response to Gn-RH injection was still high in the early cycle when the natural serum LH concentration was declining which is different from the situation in the sheep in which a Gn-RH injection after the natural preovulatory LH peak induces a much lower LH response than does injection at any other time of the oestrous cycle (Foster & Crighton, 1976b; Foster, 1978).

Table 1. Heights of LH and FSH peaks (above baseline) induced by synthetic Gn-RH injection at various stages of the oestrous cycle in the mare

Stage of cycle	Height of peak		
	LH (ng/ml)	FSH (ng/ml)	LH:FSH
Mid-cycle	195	23	8.78
(Day 10–11 after ovulation)	300	52	5.77
	102	21	4.86
	160	12	13.33
	120	7.5	16.0
Mean $\pm$ s.e.m.	175 $\pm$ 35 ^a	23.1 $\pm$ 7.8 ^a	9.74 $\pm$ 2.2 ^a
Early oestrus	270	5	54
	208	12	17.3
	145	6	24.17
	230	1.0	230
Mean $\pm$ s.e.m.	213 $\pm$ 26 ^a	6.0 $\pm$ 2.27 ^a	81.37 $\pm$ 50.1 ^{a,b}
Late oestrus	660	7	94.29
	808	6	134.66
	390	13	30
Mean $\pm$ s.e.m.	619 $\pm$ 122 ^b	8.7 $\pm$ 2.2 ^a	86.3 $\pm$ 30.5 ^b
Early cycle	320	11	29.09
(Day 5–6 after ovulation)	794	50	15.8
	154	4.5	34.22
	905	41	22.07
Mean $\pm$ s.e.m.	543 $\pm$ 181 ^{a,b}	26.2 $\pm$ 11.1 ^a	25.29 $\pm$ 4.02 ^b

In each column means with a common superscript are not significantly different ($P > 0.05$).

The LH:FSH ratio was significantly lower in terms of both height and area, in mares at mid-cycle compared with those at late oestrus and early in the cycle (Table 1, Text-fig. 1b). The large difference between the mean ratios at mid-cycle and early oestrus failed to reach significance because of the high standard error of the latter.

The dose of Gn-RH used may be important in determining the relative changes in gonadotrophin response during the cycle. In women, Wang, Lasley & Yen (1976) have shown differences in the magnitude and pattern of gonadotrophin responses to a large dose (150 µg) and a small dose (10 µg) of Gn-RH during three phases of the menstrual cycle. Thus the results of this type of experiment must be interpreted with caution.

The cyclic mare has an unusual plasma gonadotrophin profile in that there is a large FSH surge during late dioestrus when LH is at its lowest; also FSH is declining to low levels during oestrus at times when LH is still high (Text-fig. 1a). However the times of maximum responsiveness to Gn-RH for each gonadotrophin coincide with these peaks of the normal cycle (Text-fig. 1b; Table 1). This suggests that a cyclic change in pituitary sensitivity to a single releasing hormone could cause the differential release of FSH and LH that occurs during the natural oestrous cycle.

We thank the National Pituitary Agency, NIMDD, Bethesda, Maryland, U.S.A., for human FSH and LH for iodination and anti-human FSH and LH antiserum; Dr L. Nuti, University of Wisconsin, U.S.A., for equine FSH and LH; Hoechst, Frankfurt (M), Germany, for Gn-RH; I.C.I., New Zealand, for prostaglandin analogue; The Medical Unit, The Princess Margaret Hospital, Christchurch, for assistance with hormone iodination and Bruce Lawson, Joyce Harris and Darrylyn Cummings for their technical assistance.

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Received 6 November 1978