The LH response of pregnant cows and their calves to synthetic LH-RH

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Since the discovery of LH-RH and its subsequent synthesis, the study of pituitary function in various animals at all stages of reproductive life has become possible. Schally *et al.* (1971) showed that administration of natural or synthetic LH-RH released LH and FSH from the pituitary gland in animals of various species. Mauer & Rippel (1972) have shown that synthetic LH-RH (referred to by them as Gn-RH) was active in mature heifers.

In this study six mature dairy cows (4 Ayrshire, 2 Friesian) that were 7–8 years old were used. Their pituitary response, as measured by circulating LH levels, to 500 µg LH-RH given i.v. was determined on 2 consecutive days between 16 and 32 days before parturition. This dose of LH-RH corresponded to approximately 1 µg/kg body weight. Preliminary experiments in other cows have shown that this dose consistently caused LH release. Catheters were inserted into the jugular veins the day before an experiment to facilitate administration of LH-RH and withdrawal of blood. LH-RH was given in 10 ml 0.9% saline and blood samples (10 ml) were taken 10 min before, and at 10, 20, 30, 45, 60 and 90 min, and 2, 3, 4, 5 and 6 hr after injection.

The six cows gave birth to 6 bull calves and 2 heifers. The mean ($\pm$ S.E.M.) weight of the calves at 24 hr was 45.2 $\pm$ 5.8 kg. The response of 6 of these calves (4 male, 2 female) to 50 µg LH-RH injected i.v. in 1 ml 0.9% saline (about 1 µg/kg body wt) was determined within 30 hr of birth. Blood samples (10 ml) were taken from the jugular vein using heparinized Vacutainers at the same intervals as for the mothers.

Blood samples were centrifuged, plasma separated and stored deep-frozen until assayed for LH. This was measured by an established solid-phase radioimmunoassay system (Symons, Cunningham & Saba, 1974). The antiserum was raised in rabbits with bovine LH (NIH-B4) and was used at a final dilution of 1:8000; bovine LH (NIH-B8) was used for iodination and standards. Iodination was performed using the enzymatic method of Marchalonis (1969). The iodinated hormone was purified on a Sephadex G-50 column and stored deep-frozen. Before each assay further purification was achieved with cellulose adsorption chromatography as described by Jeffcoate (1971).

All the cows responded to LH-RH, although the response in Cow 6 was very small (Table 1). Plasma LH levels before LH-RH injection were below 0.5 ng/ml and rose to peak values after 1½–2 hr. Levels had returned to near preinjection values by 6 hr. These results compare favourably with those of Mauer & Rippel (1972) who showed that i.m. injection of 100 and 250 µg LH-RH in mature heifers gave peaks of LH (26 and 88 ng/ml respectively) after 120–150 min. Kittok, Britt & Convey (1972) also found peak levels of LH (25.3 $\pm$ 9.5 ng/ml) 120 min after i.v. injection of 100 µg LH-RH in cows. The variation in peak levels attained in the present experiments was however enormous, the range being 0.7–13.3 ng/ml plasma. Despite this large variation, the peak levels were lower than those reported in non-pregnant animals after a smaller dose of LH-RH by Mauer & Rippel (1972) and Kittok *et al.* (1972), although comparisons with these results should be treated with caution because of the differences in assay procedures and standards used. It does seem, however, that the pregnant cow may become refractory to LH-RH during pregnancy as has been reported for the sheep (Chamley, Findlay, Jonas, Cumming & Goding, 1974).

Although the number of Friesian cows used in the experiment was only two, both gave relatively small responses compared to the four Ayrshire cows. There was no correlation between the size of the LH response and the time before parturition that LH-RH was administered. When exactly the

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Table 1. The LH response (ng/ml) of 6 pregnant cows to 500 µg LH-RH given i.v. at zero time

Time	Cows*					
	1	2	3	4	5	6
-10 min	0.2	0.3	0.1	0.3	0.4	0.1
10 min	0.2	0.6	0.1	0.3	0.6	0.1
20 min	0.4	0.7	0.2	0.3	0.6	0.1
30 min	1.0	1.2	0.3	0.4	0.6	0.1
45 min	2.7	2.2	1.4	1.0	0.9	0.2
1 hr	6.3	6.8	3.0	2.2	1.5	0.3
1½ hr	12.6	10.3	5.3	4.6	2.2	0.4
2 hr	13.3	11.4	6.6	6.0	1.9	0.7
3 hr	8.2	8.0	4.2	3.3	1.8	0.3
4 hr	3.4	2.9	1.3	1.0	1.1	0.2
5 hr	1.2	1.0	0.2	0.4	0.6	0.1
6 hr	0.4	0.7	0.2	0.2	0.6	0.1

* 1, Ayrshire, 22 days *prepartum*; 2, Ayrshire, 20 days *prepartum*; 3, Ayrshire, 32 days *prepartum*; 4, Ayrshire, 28 days *prepartum*; 5, Friesian, 32 days *prepartum*; 6, Friesian, 16 days *prepartum*.

same experimental procedure was carried out the following day a decreased response was obtained in all 6 cows, suggesting that the first 500 µg LH-RH had either reduced the available stores of pituitary LH or had reduced the sensitivity of the pituitary to LH-RH. The injections of LH-RH within 16–32 days *prepartum* had no effect on the expected calving date and all cows gave birth normally.

Table 2 shows the mean LH response obtained after i.v. injection of 50 µg LH-RH into 4 bull calves and 2 heifers when 24–30 hr old. There was no significant difference between the response in male and female calves at this age; peak levels of LH were less varied and at a different time (30 min after injection compared to 1½–2 hr) in the calves than in their mothers. The pituitary of the newborn calf was therefore capable of responding to exogenous LH-RH more rapidly than were pregnant cows to the equivalent dose. This quicker response to LH-RH in newborn calves is similar to that observed in gonadectomized sheep of both sexes. Saba, Symons & Drane (1974) reported peak LH levels at 20 min after 25 µg LH-RH was given i.v. to castrated rams. Galloway & Pelletier (1975) also found that 100 µg LH-RH given i.v. to intact and castrated rams gave LH peaks at 120 and 29 min, respectively. LH peaks occurred at 120 min in intact ewes and at 20–40 min in ovariectomized animals after administration of 12.5 or 100 µg LH-RH (Rippel, Moyer, Johnson & Mauer, 1974).

Table 2. The mean ($\pm$ S.E.M.) LH response of six 24–30-hr-old calves (4 male, 2 females) to an i.v. injection of 50 µg LH-RH at zero time

Time	LH (ng/ml)	Time	LH (ng/ml)
-10 min	0.5 $\pm$ 0.2	2 hr	3.2 $\pm$ 0.4
10 min	6.1 $\pm$ 0.9	3 hr	1.5 $\pm$ 0.4
20 min	8.0 $\pm$ 0.6	4 hr	0.7 $\pm$ 0.3
30 min	9.6 $\pm$ 1.6	5 hr	0.6 $\pm$ 0.3
45 min	8.7 $\pm$ 0.8	6 hr	0.5 $\pm$ 0.3
1 hr	6.1 $\pm$ 0.7		

It has been shown in the present study that pregnant cows and their calves responded to LH-RH treatment, but the time course of the response was different, the newborn calves with undeveloped gonads responding in a similar way to castrated animals. Peak levels of LH after LH-RH treatment

in the pregnant cows and their calves were lower than those reported for the mature non-pregnant animal, suggesting pituitary refractoriness during the later stages of pregnancy.

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