A priming effect of LH-RH on the pituitary in rams

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In the female rat a single injection of LH-RH causes a rapid release of LH and primes the pituitary so that a further injection 1 h later produces a significantly increased response (Aiyer, Chiappa & Fink, 1974). A similar, although not so well pronounced, phenomenon occurs in ewes (Crichton & Foster, 1977) and women (Rommler & Hammerstein, 1974; Olivier & Faure, 1976; Wang, Lasley, Lein & Yen, 1976). Such a priming effect of LH-RH on LH has not been reported in male domestic animals; we therefore investigated the possibility in rams.

Seven mature, sexually active Merino and 2 Border-Leicester rams were used. The animals were kept in controlled conditions indoors and the experiment was conducted in November (spring). Blood samples were collected into heparinized tubes at 10-min intervals and injections were made through indwelling catheters placed in the jugular vein. Plasma samples were stored at -20°C until assayed for LH.

Three blood samples were collected before giving the first intravenous injection of $1\text{ }\mu\text{g}$ LH-RH (Lutal, Hoechst A. G.) dissolved in 1 ml 0.154 M-NaCl (saline). Sampling continued for 1 h and a second injection of $1\text{ }\mu\text{g}$ LH-RH was given. Samples were then collected for a further 2 h.

The response of 5 experimental Merino rams treated with two injections of LH-RH was compared with the response of 4 control rams treated with saline as follows. In one of two Merino (M) rams saline was used instead of the first LH-RH injection. In the other, examined simultaneously, saline replaced the second LH-RH injection. Two Border-Leicester (BL) rams were also used as controls with saline replacing the first LH-RH injection on one occasion and the second LH-RH injection on another. No breed differences were noted in response to these injections and the results are combined in Table 1 to give 3 control rams (2 BL, 1 M) for each treatment.

Plasma LH concentration was measured by a double-antibody radioimmunoassay method similar to that of Hopkinson, Pant & Fitzpatrick (1974) except that goat anti-equine gamma-globulin serum was used instead of the rabbit preparation and NIH-LH-S18 was used as a standard. All samples were measured in a single assay in which the within-assay variation was 4%. The plasma LH concentration and the precision of the assay were calculated by the computer programme described Burger, Lee & Rennie (1972). The variations in plasma LH concentration over time (Text-fig. 1) were calculated for each ram, the area under the curve (ng LH/ml/60 min) being estimated by the trapezoidal rule (Normand & Fortier, 1970). The area under the first curve (Text-fig. 1) represented the total increment in LH after the first injection because basal pretreatment levels in all experimental rams were below the sensitivity of the assay (0.30 ng/ml). The increments in LH during the 60 min after the second injection were calculated from the areas under the second curve (Text-fig. 1) by subtraction of the estimated residual response from the first injection. The residual areas were found by extrapolation of the first curve to the base line. The mean LH response to $1\text{ }\mu\text{g}$ LH-RH or saline during the 60 min after each injection were compared (Table 1) by using the paired *t* test.

Table 1. Effect of two injections of $1\text{ }\mu\text{g}$ LH-RH 1 h apart on plasma LH levels in intact rams

No. of rams	Treatment		Mean ($\pm$ S.E.M.) response*		Significance of difference
	1st injection	2nd injection	1st injection (0–60 min)	2nd injection (60–120 min)	
5	LH-RH	LH-RH	4.23 ± 1.01	10.49 ± 1.01	$P < 0.01$
3	Saline	LH-RH	0.54 ± 0.16	4.44 ± 0.58	$P < 0.001$
3	LH-RH	Saline	3.45 ± 0.74	0.78 ± 0.12	$P < 0.001$

* Areas under curve of LH concentration over time (ng LH/ml/60 min).

Text-fig. 1. Plasma LH levels in 5 Merino rams injected intravenously (arrows) with two 1 µg doses of LH-RH 1 h apart.

The mean LH response to the second injection of LH-RH was significantly ($P < 0.01$) greater than the response to the first (Table 1; Text-fig. 1). The 5 experimental animals showed the same pattern of response, but the sensitivity of individual animals to the treatment differed (Text-fig. 1). When the first injection of LH-RH was replaced by saline in the control rams the response to LH-RH was no greater than to a single LH-RH injection (Table 1). Similarly, the saline injection following LH-RH treatment produced no significant increment in LH (Table 1). A single small dose of LH-RH administered intravenously to the ram therefore has the dual action of releasing some LH and sensitizing the pituitary so that greater amounts of LH are released by a second dose of LH-RH 1 h later.

In rams, biphasic patterns of LH response to a single large dose of LH-RH (Arimura, Debeljuk, Matsuo & Schally, 1972) and to infusions (Bremner, Findlay, Cumming, Hudson & de Kretser, 1976) have been reported previously. The priming effect demonstrated here by using two, much smaller, doses of LH-RH 1 h apart could present a clearer picture of the biphasic patterns of LH release. It is postulated that the first dose of LH-RH discharges some of the acutely releasable LH (equivalent to the first pool in the biphasic type of response; Bremner & Poulsen, 1974) and sensitizes the pituitary gland to further treatment with LH-RH. In female animals a significantly greater release of LH in response to the second injection was found to be dependent on oestrogen and progesterone levels and protein and RNA synthesis (Aiyer *et al.*, 1974; Pickering & Fink, 1976a,b). The factors influencing the magnitude of the second, greater LH response to LH-RH in male animals remain obscure. The mechanism of the phenomenon in rams and its physiological significance are the subject of further study.

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