Pituitary LH levels and response to LH-RH in female rats pretreated with oestradiol

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The pituitary response to LH-RH can be modified by sex steroids (Debeljuk, Arimura & Schally, 1972a; Debeljuk, 1973; Debeljuk, Vilchez Martínez, Arimura & Schally, 1974) which can affect the gonadotrophin stores in the pituitary gland (McCann & Ramírez, 1964). It has been suggested, however, that an increased or decreased response to LH-RH is not necessarily correlated with an increase or decrease of pituitary gonadotrophin concentrations (Debeljuk, Arimura & Schally, 1972b; Debeljuk, Rozados, Daskal & Villegas Vélez, 1975). We therefore attempted to correlate changes in the pituitary response to LH-RH with pituitary LH concentrations in rats.

Female rats of the Wistar strain (mean weight 200 g) were maintained on a diet of laboratory chow and vegetables, and had free access to water. The light schedule was 14 h light/24 h. The stage of the oestrous cycle was checked every day by observation of the vaginal cytology for at least 3 cycles and only rats with regular 4-day cycles were selected. Intact rats on the first day of dioestrus were allocated to groups (12 rats/group) and were killed by decapitation, after treatment, on the 2nd day of dioestrus between 15.00 and 18.00 h, 20 min after i.v. injection of 0.2 ml acidified saline (6 rats/group) or 400 ng LH-RH (kindly supplied by Dr Andrew Schally, Tulane University School of Medicine, New Orleans, Louisiana) in 0.2 ml acidified saline (6 rats/group). The animals in Group 1 were controls and received only 0.5 ml corn oil s.c. The rats in Groups 2, 3 and 4 received a s.c. injection of 20 µg oestradiol benzoate (Sigma Chemical Company) in 0.5 ml corn oil at 3, 6 and 24 h respectively before the LH-RH injection. After ovariectomy 10 days previously, a further 48 rats (Groups 5, 6, 7 and 8) were treated as described above for intact rats.

At death, blood was collected from the trunk and serum was separated by centrifugation and kept frozen until assayed. Pituitaries were quickly extirpated and after removal of the neural lobe were weighed. Anterior pituitary tissue was kept frozen until homogenized in saline and assayed for LH by the double-antibody method described by Niswender, Midgley, Monroe & Reichert (1968). An ovine LH preparation was used for labelling with ^{125}I ; the first antibody was an anti-ovine LH serum and NIAMDD-Rat LH-RP1 was used as the standard preparation.

The significance of the differences among groups was checked by means of Student's *t* test and Duncan's new multiple range test (Steel & Torrie, 1960).

Results

As shown in Table 1, serum LH levels were increased in the dioestrous and ovariectomized rats injected with LH-RH compared with those receiving saline only, although the magnitude of the response varied with the duration of oestradiol pretreatment. Pituitary LH concentration in the saline-injected intact animals 24 h after pretreatment with oestradiol benzoate (Group 4) was significantly lower ($P < 0.05$) than in the rats in the other two groups receiving saline and oestradiol (Groups 2 and 3). In the ovariectomized rats, pituitary LH concentrations in saline-injected rats were significantly different ($P < 0.05$) from those in LH-RH-injected rats only in the group treated with oestradiol benzoate for 24 h (Group 8).

From these results it is evident that the changes in the magnitude of the pituitary response to LH-RH that were seen from 3 to 24 h after the injection of oestradiol were not accompanied by concomitant changes in pituitary LH concentrations in intact dioestrous rats. This was also true for ovariectomized rats, except for those receiving oestradiol for 24 h: their response to LH-RH was maximal when the lowest pituitary LH concentration was noted. Although pituitary responsiveness

Table 1. Mean $\pm$ S.E.M. serum and pituitary LH concentrations in dioestrous or ovariectomized rats (6/group) after the injection of saline or 400 ng LH-RH after pretreatment with oestradiol benzoate (OB) for various times

	Corn oil	OB-3 h	OB-6 h	OB-24 h
<i>Dioestrous rats—Group</i>	1	2	3	4
Serum LH (ng/ml)				
Saline	8.98 $\pm$ 2.41	0.90 $\pm$ 0.19†	6.90 $\pm$ 1.15	22.25 $\pm$ 7.31†
LH-RH	292.70 $\pm$ 113.53*	17.66 $\pm$ 3.02*†	80.00 $\pm$ 5.45*†	1266.60 $\pm$ 214.36*†
Pituitary LH (μ g/mg)				
Saline	7.86 $\pm$ 3.00	12.62 $\pm$ 2.83	12.37 $\pm$ 2.29	4.13 $\pm$ 1.05
LH-RH	9.71 $\pm$ 2.85	5.59 $\pm$ 1.38	5.95 $\pm$ 2.19	3.44 $\pm$ 1.18
<i>Ovariectomized rats—Group</i>	5	6	7	8
Serum LH (ng/ml)				
Saline	392.50 $\pm$ 191.81	66.91 $\pm$ 13.30†	54.00 $\pm$ 7.53†	90.83 $\pm$ 9.50†
LH-RH	3013.30 $\pm$ 751.42*	186.80 $\pm$ 39.47*†	477.14 $\pm$ 60.91*†	4450.00 $\pm$ 683.67*
Pituitary LH (μ g/mg)				
Saline	54.57 $\pm$ 8.94	57.22 $\pm$ 4.83	52.17 $\pm$ 5.01	55.24 $\pm$ 2.45
LH-RH	50.59 $\pm$ 7.88	56.19 $\pm$ 6.85	44.62 $\pm$ 2.91	40.17 $\pm$ 3.10*

* Significantly different from value for respective saline-injected animals, $P < 0.05$.† Significantly different from value for respective oil-injected animals, $P < 0.05$.

to LH-RH can be modified by oestradiol it is evident that, at least under the conditions of the present experiment, the response, as determined by LH release, can be inhibited or augmented regardless of any apparent change in pituitary LH concentrations.

Previous reports have also suggested that an increment of the pituitary response to LH-RH may not be due simply to an increase in pituitary LH stores (Debeljuk *et al.*, 1972b, 1975). A change, too subtle to be detected by the assay methods employed, in the pituitary content of a readily releasable pool of LH (Bogdanove & Gay, 1967) could affect the pituitary response to LH-RH. Such a pool could constitute only a small part of the total pituitary LH content, a suggestion supported by findings of a lack of detectable pituitary LH depletion even after large doses of LH-RH (Debeljuk *et al.*, 1972b). Evidence has also been produced for the existence of at least two pools of LH in the human pituitary, one responding quickly to LH-RH and the other requiring a more prolonged stimulation (Bremner & Paulsen, 1974).

We thank Dr Niswender, Dr L. E. Reichert and NIAMDD, Bethesda, for gifts of the reagents used in the radioimmunoassays. This work was supported by a grant of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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Received 22 December 1976