

POSSIBLE NEGATIVE ULTRA-SHORT LOOP FEEDBACK OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IN THE OVARIECTOMIZED RAT

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Abstract. To determine if LHRH might act within the brain to modify its own release, repeated blood samples were removed from conscious ovariectomized rats and minute doses of LHRH were injected into the third ventricle (3V). The effect of these injections on plasma LH and FSH was measured by radioimmunoassay (RIA). The higher dose of intraventricular LHRH (10 ng in 2 μ l) induced an increase in plasma LH within 10 min after its injection. Plasma LH decreased for the next 60 min. This was followed by restoration of LH pulses characteristic of the ovariectomized rat. This dose of LHRH slightly elevated plasma FSH concentrations. In stark contrast, a 10 fold lower dose of 1 ng of LHRH injected into the ventricle resulted in a highly significant decrease of plasma LH at 10 min following injection, followed by return of LH pulsations. There was no effect on the pulsatile release of FSH. The results are interpreted to mean that at the higher dose, sufficient LHRH reached the site of origin of the hypophyseal portal vessels in the median eminence so that it diffused into portal vessels and was delivered to the gonadotrophs to induce LH release. In contrast, the lower dose provided sufficient hypothalamic concentrations of the peptide to suppress the discharge of the LHRH neurons, thereby leading to a decline in plasma LH, indicative of an ultrashort-loop negative feedback of LHRH to suppress its own release. © 1985 Society for Experimental Biology and Medicine.

We have recently obtained evidence for negative ultrashort-loop feedback of some hypothalamic releasing and inhibiting hormones to suppress their own release. For example, injection of somatostatin into the 3rd ventricle (3V) elevated instead of lowering growth hormone (GH) release (1) which indicates that high local concentrations of the peptide in the vicinity of the ventricle either inhibited somatostatin release or enhanced GH-releasing factor (GRF) discharge or acted by a combination of these actions to stimulate GH release from the adenohypophysis. Similarly, we have demonstrated that intraventricular administration of low doses of GRF results in a depression instead of an ele-

vation of GH levels in plasma which indicates that GRF exerts a similar negative ultrashort-loop feedback (2). The present experiments were designed to determine if LHRH exerted a negative ultrashort-loop feedback to inhibit the enhanced pulsatile LH release in the castrate rat.

Materials and Methods.

Animals. Female rats (180-200 g) of the Sprague-Dawley strain (Holtzman, Madison, WI) which were housed in group cages at a temperature of 24 \pm 2 C with lights on from 0500-1900h were used throughout. They had free access to rat chow and water.

After the rats had shown 2 to 3 consecutive 4 day estrous cycles, the

animals were ovariectomized while anesthetized with ether. They were used for experiment 21 days after ovariectomy.

Experimental procedure. One week before the experiment, while the rats were anesthetized with tribromoethanol (ip), a 23 gauge stainless steel cannula was implanted into the 3V (3). Subsequently, the animals were housed in individual cages. Only rats that presented a continuous flow of cerebrospinal fluid after removal of the stylette of the 3V cannula were used. Twenty-four hours before the experiment, the rats were submitted to right external jugular vein cannulation as described by Harms and Ojeda (4), while anesthetized with ether. The animals were then returned to the animal quarters overnight. On the day of experiment, all animals were brought to the laboratory and kept undisturbed for at least 2 hours. After that blood samples (250 μ l) were withdrawn every 10 min during 3 1/2 hours. After removal of each sample, an equal volume of saline (0.9% NaCl) was replaced. The blood was placed on ice (4 C) and after the final collection, the samples were centrifuged at low speed for 15 min. The plasma was separated and stored (-20 C) prior to RIA of plasma FSH and LH.

Treatments. After 1 1/2 hours (control bleeding period) a saline solution containing LHRH (Peninsula Laboratories) in different doses in a volume of 2 μ l was delivered during 30 sec into the 3V using a Hamilton microliter syringe. The animals were divided into three groups: 1) saline control group, 2) LHRH in a concentration of 10 ng/2 μ l and 3) LHRH in a concentration of 1 ng/2 μ l.

Radioimmunoassays. Plasma LH was measured by the method of Niswender et al. (5) and the results were expressed in terms of NIH-LH-S1 standard to be comparable with previous results from this laboratory. FSH concentrations were measured by the RIA kits supplied by NIADDK and results expressed in terms of the FSH-RPI reference preparation. The volume of plasma assayed for both FSH and LH was 20 μ l. The sensitivity of the FSH and LH assays was 32 and 0.5 ng/ml, respectively. All samples were run in the same assay in duplicate; interassay variation was 8.9

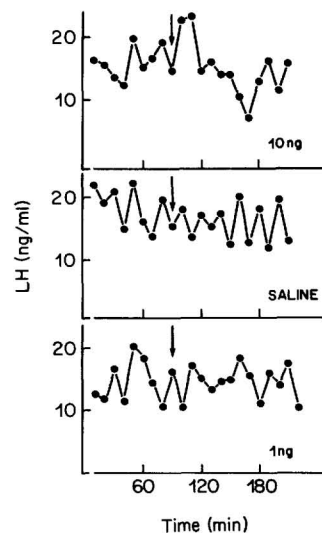


Fig. 1 Examples of the effects of intraventricular (3V) injection of LHRH or saline on the pattern of plasma LH in individual animals.

and 8.5% for the LH and FSH assays, respectively.

Statistics. Differences between the pre-injection (after 90 minutes of sampling every 10 min) and post-injection samples were determined by paired "t" test and also calculated by analysis of variance with repeated measure followed by the Student-Newman-Keuls test.

Results.

Control injections of saline intraventricularly. The pattern of pulsatile release of FSH and LH characteristic of ovariectomized female rats was not altered by the intraventricular (3V) injection of saline in any of the rats. An example of the effect of saline on the pulsatile pattern of LH is illustrated in Fig. 1.

Effect of intraventricular injections of LHRH on plasma LH and FSH concentrations. The intraventricular injection of LHRH at a dose of 10 ng produced a significant increase in plasma LH ($p < .05$) within 10 min following the injection (Fig. 2). The values began to decline at 20 min and had returned to basal levels by 60 min. Examination of the results from individual animals indicated that there was a transient elevation in LH at 10 and usually 20 min after the injection,

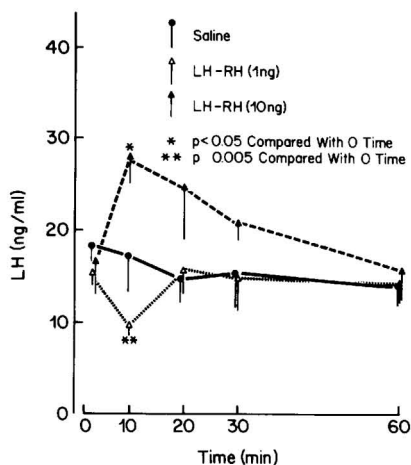


Fig. 2 The effects on plasma LH of intraventricular injection of saline or LHRH. Values are mean \pm 1 SEM; n=6 for each group.

followed by a cessation of pulsatile release of LH for approximately the next 60 min. This dose of LHRH slightly elevated plasma FSH ($p < 0.05$) in the 10 min immediately following its injection (Fig. 3).

In striking contrast, the intraventricular administration of the lower dose of LHRH (1 ng) decreased plasma LH significantly ($p < 0.005$, paired t test) within 10 min on comparison with the values immediately

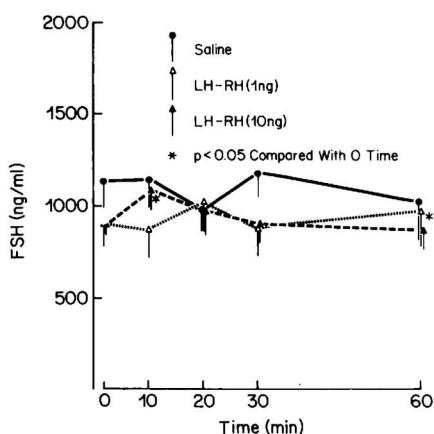


Fig. 3 The effects on plasma FSH of intraventricular injection of saline or LHRH. Values are mean \pm 1 SEM; n=6 for each group.

prior to injection (Fig. 2). The concentrations of LH then returned to control by 20 min and remained unchanged for the remainder of the experimental period (60 min) (Fig. 2). The decrease at 10 min was achieved by a fall in plasma LH below the pre-injection level in every animal. Following this acute decline pulsatile LH secretion resumed (Fig. 1). There was no effect of this lower dose of LHRH on the pattern of pulsatile FSH secretion (Fig. 3).

Discussion

It was shown previously that relatively large doses of LHRH injected into the 3 V increase plasma LH levels (6). Presumably the peptide is taken up by the hypothalamic tissue adjacent to the 3V, diffuses to the vicinity of the hypophyseal portal vessels which transport it to the pituitary to stimulate the release of LH from the gonadotrophs. Similar results were obtained in the present experiments with the higher, 10 ng, dose of LHRH in the first minutes after injection. When the dose of LHRH injected into the ventricle was reduced 10-fold to 1 ng, a reduction instead of an increase in plasma LH occurred. This was followed by a return to control values. Presumably in this situation the concentration of LHRH which reaches the pituitary is too small to stimulate the gonadotrophs; however, it is high enough in periventricular tissue to inhibit the release of LHRH from the LHRH neurons, which results in a decline in plasma LH. The inhibition is shortlived and LH release quickly returns to normal. This action may occur by means of recurrent collaterals of LHRH neurons which synapse on the perikarya of these neurons and inhibit their discharge. The phenomenon of recurrent inhibition within the tuberoinfundibular region, whereby axon collaterals return to and synapse on the cell bodies of origin to inhibit their firing, has been described (7). Kozłowski et al (8) have observed recurrent collaterals of LHRH neurons synapsing on the cell bodies of the same neurons in electron micrographs. Consequently, our data support the suggestion that recurrent

inhibition of LHRH neurons; i.e., negative ultrashort-loop feedback may play a role in terminating the pulses of LH release in castrate and possibly even in normal animals. The cessation of pulsations of LH which followed the higher dose of LHRH from 20 to 90 mins after the injection could reflect negative ultrashort-loop feedback of LHRH, active during this period and not masked by a pituitary site of action, and/or be the result of the prior release of LH which immediately followed the intraventricular injection of this dose of the peptide.

The higher dose of LHRH had only a minimal effect to elevate plasma FSH in a few rats and there was no effect of the lower dose. This reflects the lesser potency of LHRH to stimulate FSH release. The lower dose failed to inhibit FSH release which could be because it failed to block the release of FSH-releasing factor (FSHRF)(9) and/or that the inhibition of LHRH release was so transient that there was no significant decline in plasma FSH in view of its longer half-life in the circulation (10).

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