BINDING CAPACITY OF LUTEINIZING HORMONE-RELEASING HORMONE AND ITS ANALOGUES FOR PITUITARY RECEPTOR SITES

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SUMMARY

Competition for luteinizing hormone-releasing hormone (LH-RH) receptor sites by the inhibitory analog [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH and by the superactive stimulatory analog [D-Trp^6]-LH-RH was observed in adenohypophysial homogenates incubated at 4°C. Competition for LH-RH binding sites was less evident with adenohypophysial plasma membranes. The binding affinities of these analogues to LH-RH pituitary receptors can explain at least in part their respective action in blocking ovulation and in inducing a greater release of luteinizing hormone and follicle stimulating hormone than the parent hormone.

The mechanism of action of LH-RH in inducing LH and FSH release from the pituitary gland is not completely clear, but the first step may be the binding to the pituitary receptors. Several antagonistic analogues of LH-RH, injected on day of proestrus, are able to inhibit the LH surge and block ovulation in rats (1,2) and in other mammals (3). Thus [D-Phe^2, D-Trp^3, D-Phe^6]- LH-RH, one of the best antagonists, injected either at 9 a.m. (1.5 mg/rat), or at noon (1 mg/rat), suppressed ovulation in proestrous rats by 67% and 70%, respectively (4). On the other hand, stimulatory superactive analogues of LH-RH have the capacity to cause a prolonged increase in the release of LH and FSH. For instance, [D-Trp^6]-LH-RH is 21 and 13 times more active in LH and FSH release, respectively, than LH-RH in the immature male rat assay (5). The mechanism of action of these analogues of LH-RH is also unknown. Because these peptides could modify the binding of LH-RH to

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; APM, adenohypophysial plasma membranes.

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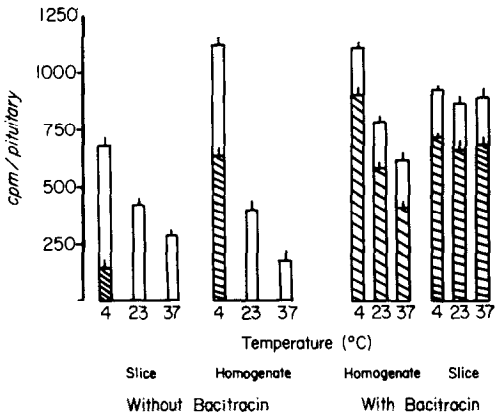

Fig.1: Uptake of ^{125}I-LH-RH by rat anterior pituitaries. The tissues were incubated with 27000 cpm of ^{125}I-LH-RH for 30 min at different temperatures. Each bar is the mean of 4 replicates. Bacitracin: 0.5 mg/ml of medium. Open bars represent the total uptake of ^{125}I-LH-RH and the shaded bars the uptake after subtracting the blank values.

the pituitary receptors, we have studied the in vitro effect of the two typical analogues mentioned above, on the binding of ^{125}I-LH-RH to pituitary in homogenates or in purified APM obtained from female rats.

METHODS

The anterior pituitaries were homogenized in Hepes buffer (mM:NaCl, 137; KCl, 5; Na_2HPO_4, 0.7; N-2 Hydroxyethylpiperazine-N-2-ethane sulfonic acid, 25; $MgCl_2$, 5; sucrose, 300) or cut into slices. The homogenates or slices were placed in glass tubes (10 x 75mm) coated with a solution of 1% gelatin. Each tube contained homogenate or slices equivalent to one pituitary in 0.5 ml of buffer and 25 µl of 4.5 nM ^{125}I-LH-RH (300 µCi/µg). LH-RH was iodinated following the lactoperoxidase method described by Arimura et al.(6). The tubes were incubated for 30 min at different temperatures (4, 23, and 37°C) with or without Bacitracin (0.5 mg/ml), a compound that inhibits the in vitro degradation of LH-RH (7). Tubes without tissue containing only 25 µl of iodinated LH-RH were used as blanks. The incubation was stopped by adding 2 ml of the same cold buffer and centrifuging immediately. After washing the slices or the pellet of homogenate twice and discarding the supernatant, the radioactivity was counted in a gamma counter. The results were corrected by subtracting the blank values.

To prepare APM, 100 anterior pituitaries obtained from 15 day ovariectomized adult rats were homogenized in 0.3 M sucrose, 10 volumes (w/v), and centrifuged at 850 g for 12 min in a refrigerated centrifuge. The pellet was resuspended in an appropriate volume of 0.3 M sucrose and adjusted with 2.0 M sucrose to 1.58 M. Aliquots of this suspension were transfered to Spinco SW27 tubes and covered with a layer of 0.3 M sucrose. After centrifuging at 80000 g for 1 h, a band containing the membranes was obtained in the supernatant. This fraction was collected, diluted with distilled water to 0.3 M sucrose concentration and

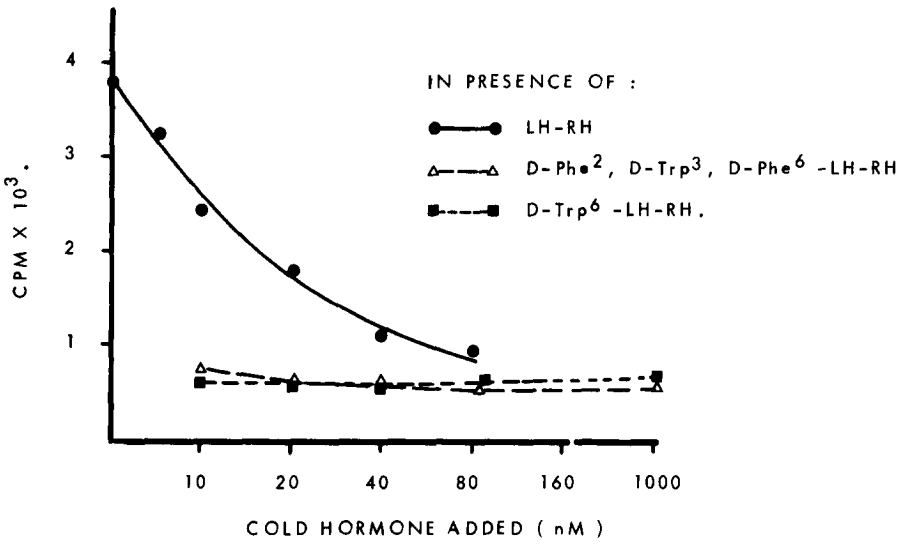

Fig.2: Effects of LH-RH and its analogues on the binding of ^{125}I-LH-RH by pituitary homogenates. The anterior pituitary was homogenized in Hepes buffer pH 7.2. The homogenate equivalent to 1 pituitary in 0.5 ml was incubated with 25 µl of a solution 4.5 nM of ^{125}I-LH-RH (300 µCi/µg) for 30 min and at 4°C in presence of LH-RH, [D-Phe^2, D-Trp^3, D-Phe^6] -LH-RH, or [D-Trp^6]-LH-RH. Each point represents the average of triplicate experiments.

centrifuged again at 1000 g for 12 min. The pellet was washed once with 0.3 M sucrose and then with 1 ml of 0.17 M NaCl plus 3 ml of 0.2 M $NaHCO_3$, pH 7.8 (8). The final pellet contained a high concentration of membranes (75-80%) as was confirmed under the electron microscope. Aliquots of APM containing 50 µg of protein were incubated for 30 min at 4°C with ^{125}I-LH-RH and cold LH-RH or its analogues, as described above.

When pituitary homogenate and slices were incubated without Bacitracin the bound tracer was only observed in the tubes incubated at 4°C, indicating that the LH-RH was degraded to a greater extent at 23°C and 37°C (Fig.1). However, binding of ^{125}I-LH-RH was observed at all temperatures studied when Bacitracin was added to the incubation media (Fig.1). These results demonstrated that Bacitracin can inhibit the in vitro degradation of LH-RH by the pituitary gland, as it does in brain tissues (7). The best experimental conditions for the binding of iodinated LH-RH were found to consist of the incubation of homogenate with Bacitracin for 30 min at 4°C. Under these conditions the effect of LH-RH analogues on the pituitary binding for ^{125}I-

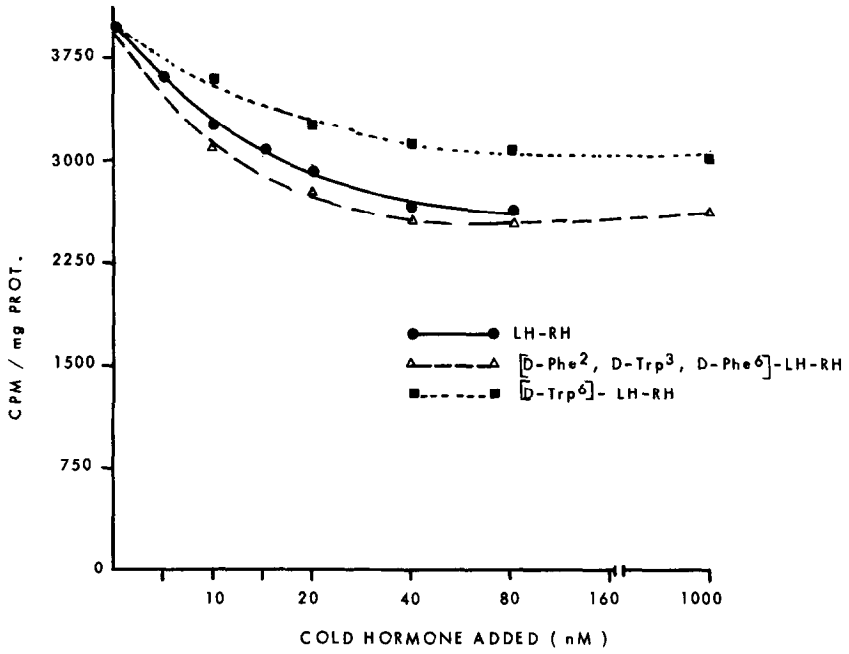

Fig.3: Effects of LH-RH and its analogues on the binding of ^{125}I-LH-RH by APM. Fifty µg of APM protein was incubated with 25 µl of a solution 5 nM of ^{125}I-LH-RH in presence of either LH-RH, [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH, or [D-Trp^6]-LH-RH for 30 min at 4°C. Each point represents the average of triplicate experiments.

LH-RH was tested. The homogenate was incubated with 25 µl of iodinated LH-RH (5 nM), in the presence of 25 µl of different concentration of either cold LH-RH (5 to 80 nM), or [D-Trp^6]-LH-RH or [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH (10 to 1000 nM). As shown in Fig.2, LH-RH competed for the receptor sites with the tracer, depending on cold hormone concentration in the incubation media. Both analogues, at the same concentration as LH-RH, displaced the ^{125}I-LH-RH from the receptor sites to a greater degree than did the parent hormone.

Displacement of iodinated LH-RH by cold LH-RH in the APM was less than that in the homogenate (Fig.3). Competition by LH-RH in the APM was less than that produced by [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH, but greater than that by [D-Trp^6]-LH-RH (Fig.3). The difference in patterns observed in the homogenate and in the APM could be explained by the unique receptor sites for

LH-RH that exist in the APM, in contrast to the homogenates where other cellular structures, such as granules (9), which also can bind ^{125}I-LH-RH may be present. Although we have estimated the number of binding sites and the affinity constant for binding of LH-RH by APM (10), it could not be excluded that during the preparation of APM the LH-RH receptors were partially destroyed. Both the agonist and antagonist used in this study have the capacity to displace LH-RH and also probably have a higher binding affinity than LH-RH for its pituitary receptors. Thus, the greater and longer-acting effect of the superactive analogues of LH-RH could be due to that property. Similarly, the blockade of the ovulation induced by several antagonists of LH-RH may be explained by the same mechanism of binding to LH-RH pituitary receptors. In this way these inhibitory analogues are able to suppress the physiological release of gonadotropins responsible for ovulation.

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