RESISTANCE TO ENZYMIC DEGRADATION OF LH-RH ANALOGUES POSSESSING INCREASED BIOLOGICAL ACTIVITY

Y. Koch and Tallie Baram*
Department of Hormone Research

E. Hazum* and M. Fridkin
Department of Organic Chemistry

The Weizmann Institute of Science, Rehovot, Israel

Received October 29,1976

SUMMARY

Three analogues of LH-RH in which Dextrarotatory amino acids were substituted for the Gly^6, and two additional analogues in which the Leu^7 residue was also modified, were subjected to enzymic preparations derived from rat hypothalamus or anterior pituitary. These enzymes, known to cleave LH-RH, preferentially at the Gly^6-Leu^7 position, proved less effective in degrading all the analogues tested. Among the Gly^6 substituted analogues, [D-Trp^6] LH-RH, having the highest LH-releasing activity, was most resistant to degradation. Additional modification, at position 7, although rendering the analogues immune to enzymic attack, did not further enhance their biological potency. These data suggest that degradation of LH-RH is a physiological determinant of its biological activity and has therefore to be considered with on designing new, potent analogues of the hormone.

Luteinizing hormone-releasing hormone (LH-RH) can be rapidly degraded by potent enzymes present in rat hypothalamus and anterior pituitary (1, 2, 3). These enzymes cleave the decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly NH_2, preferentially at the Gly^6 - Leu^7 bond (1). As the degrading activity is present at the sites of release (hypothalamus) and of action (pituitary) of LH-RH, it may have a physiological role in the fine control of the amount of the hormone at the receptor site. Several synthetic analogues of LH-RH, modified at positions Gly^6 and Leu^7 have been reported to possess enhanced luteinizing hormone-releasing activities in in vitro systems (4, 5, 6). The relationship between the resistance to LH-RH - degrading enzymes and the increased biological potency of these analogues is dealt with in this study.

---

* In partial fulfilment of the requirements for the Ph. D. degree of the Graduate School of the Weizmann Institute of Science.

Table 1. Parameters of LH-RH degradation by pituitary-enzyme preparation

Enzyme (μg protein)	LH-RH recovered (ng)	LH-RH degraded (%)	Specific activity
0	560 ± 15.0	0	0
50	310 ± 37.8	44.6 ± 9.4	147.1
100	200 ± 20.8	64.3 ± 6.2	180.0
200	133 ± 7.2	76.2 ± 3.9	185.6

560 ng of LH-RH were exposed to various doses of enzyme, in a final volume of 1 ml, for 20 min at 37° C. The reaction was terminated by placing the reaction tubes in a boiling-water bath for 3 min. Tubes were spun for 10 min at 3000 x g and aliquots taken for RIA of LH-RH. Specific activity of the enzyme was defined as the amount of degraded LH-RH (ng) per log protein (μg) of the enzymic preparation. Mean ± SEM for 6 determinations.

MATERIALS AND METHODS

LH-RH and analogues. LH-RH was supplied by Hoechst, A.G., (Frankfurt); [D-Leu^6] LH-RH, [D-Ala^6] LH-RH, [D-Leu^6, (N$^{\alpha}$-Me) Leu^7] LH-RH and [D-Trp^6] LH-RH were a kind gift of Dr. D. Coy, while [D-Ala^6, (N$^{\alpha}$-Me) Leu^7] LH-RH was received from Dr. W. Vale.

Enzyme preparation. LH-RH - degrading enzyme was prepared as described previously (1). Briefly, medial-basal-hypothalami from 5 rats (about 8 mg each) or 5 anterior pituitaries (about 9 mg each), were detached immediately after decapitation and immersed in a Thomas homogenizing vessel containing 1 ml of phosphate-buffered saline (PBS, pH 6.9). The tissue was homogenized (10 strokes) by a teflon pestle and spun for 10 min at 17,000 x g (Servall RC-2, rotor head SW 24). The resulting supernatant fraction was made up to the concentration of 30 mg wet weight/ml. Protein content of the preparation was determined by the Lowry procedure (7).

Degradation reaction. 500 ng of LH-RH or of an analogue were subjected, in a final volume of 1 ml of PBS, to enzyme preparations derived from 3, 1.5 and 0.75 mg of tissue (corresponding to about 200, 100 and 50 μg protein, respectively). The reaction, lasting for 20 min at 37°C, was terminated by placing the tubes in a boiling-water bath for 3 min. Heat resistant polypropylene tubes were used, as LH-RH tends to adsorb to glass.

Determination of LH-RH and its analogues. Tubes containing LHRH or analogues were spun for 10 min at 3000 x g and aliquots of the supernatant were taken for radioimmunoassay (RIA). The RIA system for LH-RH has been described at length elsewhere (8). Separate standard curves were constructed for each analogue. Results were evaluated using Student's t-test.

Table 2. Degradation of LH-RH analogues by LH-RH - degrading enzymes of rat hypothalamus and pituitary

Analogues	Enzyme source: Hypothalamus % degradation		Enzyme source: Anterior pituitary % degradation	
[D-Ala^6] LH-RH	68.4 ± 5.3	(12)	82.2 ± 4.0	(13)
[D-Leu^6] LH-RH	54.1 ± 3.0	(21)	58.8 ± 4.8	(18)
[D-Trp^6] LH-RH	12.0 ± 4.1	(23)	17.7 ± 4.9	(25)
[D-Ala^6, (N^{α}-Me) Leu^7] LH-RH	0	(21)	0	(9)

LH-RH and analogues (500 ng) were exposed to enzyme preparations for 20 min at 37°C, as described in Table 1 and in the text. LH-RH degradation is defined as 100%, and the extent of analogue degradation is given as percent ± SEM of that of LH-RH. Value in brackets indicates number of determinations.

RESULTS

Exposure of LH-RH to increasing amounts of enzymic preparations derived from rat hypothalamus or anterior pituitary results in increasing degradation of the hormone (see Table 1 for pituitary enzyme). Under the conditions of the reaction i.e. 20 min incubation at 37°C with pituitary preparations corresponding to 100 μg protein, about 36% of 500 ng of LH-RH can be recovered (Table 2). The extent to which LH-RH is degraded is arbitrarily defined as 100%; analogue degradation under the same conditions is expressed relatively to that of LH-RH. Analogues modified at the Gly^6 or the Gly^6 - Leu^7 residues have a higher resistance to the LH-RH - degrading enzymes (Table 2); the relative resistance of the analogues is: [D-Ala^6, (N^{α}-Me) Leu^7] LH-RH > [D-Trp^6] LH-RH > [D-Leu^6] LH-RH > [D-Ala^6] LH-RH > LH-RH. [D-Leu^6, (N^{α}-Me) Leu^7] LH-RH is as resistant as [D-Ala^6, (N^{α}-Me) Leu^7] LH-RH to enzymic attack.

DISCUSSION

The factors determining the biological potencies of hormones and drugs include their affinities to their respective receptors and their local concentration at their target-sites; the latter, in turn, is dependent on the rate of clearance and the elimina-

tion by enzymes on the route to, and at the site of action. Thus, information about the relative resistance of LH-RH analogues to enzymic degradation may aid investigators in synthetizing substances possessing enhanced biological activity. We, therefore, subjected LH-RH, [D-Ala^6] LH-RH, [D-Leu^6] LH-RH, [D-Trp^6] LH-RH, [D-Ala^6, (N^{α}-Me) Leu^7] LH-RH and [D-Leu^6, (N^{α}-Me) Leu^7] LH-RH to enzymic preparations derived from rat hypothalamus or anterior pituitary.

From the results shown above, it may be concluded that a correlation exists between the increased biological potencies of LH-RH analogues and their resistance to enzymic degradation. Thus, the least active analogue (5), [D-Ala^6] LH-RH, is also the most susceptible, while [D-Trp^6] LH-RH, highly resistant to enzymic cleavage, has superior biological potency (5). [D-Ala^6, (N^{α}-Me) Leu^7] LH-RH, however, is exceptional in that, though immune to enzymic attack, it is only as active as [D-Ala^6] LH-RH (4, 6). This discrepancy may stem from impaired interaction of the analogue with its receptor; the N^{α}-methylic group may interfere with the binding to, or with the triggering of the receptor.

Acknowledgements

We are grateful to Miss N. Ben-Aroya for technical assistance and to Mr. S. Yossef for devoted animal care. The work was supported by a grant of the Ford Foundation and the Population Council Inc., N.Y., to Dr. H.R. Lindner, whom we thank for his interest in this study.

REFERENCES

1. Koch, Y., Baram, T., Chobsieng, P. and Fridkin, M., Biochem. Biophys. Res. Commun., 61, 95 (1974).

2. Griffiths, E.C., Hooper, K.C., Jeffcoate, S.L. and Holland, D.T., Acta Endocr., 77, 435 (1974).

3. Kochman, K., Kerdelhue, B., Zor, U. and Jutisz, M., FEBS Lett., 50, 190 (1975).

4. Monahan, M.W., Amoss, M.S., Anderson, H.A. and Vale, W., Biochemistry, 12, 4616 (1973).

5. Coy, D.H., Vilchez-Martinez, J.A., Coy, E.J. and Schally, A.V., J. Med. Chem., 19, 423 (1976).

6. Ling, N. and Vale, W., Biochem. Biophys. Res. Commun., 63, 801 (1975).

7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. Biol. Chem., 193, 265 (1951).

8. Koch, Y., Wilchek, M., Fridkin, M., Chobsieng, P., Zor, U. and Lindner, H.R., Biochem. Biophys. Res. Commun., 55, 616 (1973).