LUTEINIZING RELEASING HORMONE, SYNTHESIS AND ARG^8-ANALOGS, AND CONFORMATION-SEQUENCE-ACTIVITY RELATIONSHIPS

by

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Received April 10, 1972

SUMMARY

The luteinizing releasing hormone (LRH or pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) has been usefully synthesized by coupling the unprotected hexapeptide and tetrapeptide, pGlu-His-Trp-Ser-Tyr-Gly-OH and Leu-Arg-Pro-Gly-NH_2. This new versatile synthesis also conveniently provides analogs with changes in the 7-10-positions. Possible conformations of parallel planarity of Trp- and Tyr-, and significance of the guanidino group for activity and potency of LRH led to synthesis of His^8-LRH, Nva^8-LRH and Des-Arg^8-LRH. Activities indicate a participating ionic mechanism for release and potency due to a basic moiety in the 8-position. Data on Nva^8-LRH and Des-Arg^8-LRH seem to support concepts of conformation and basicity.

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LRH) has been synthesized by several groups of investigators. This decapeptide was proposed by Matsuo et al. (1) for the structure of the luteinizing releasing hormone of the hypothalamus, and they also proposed that this decapeptide is actually the LH- and FSH- releasing hormone.

Monahan et al. (2) reported their total synthesis of this decapeptide by a solid-phase technique, and the hormonal potencies of the product.

Sievertsson et al. (3) described two syntheses of this decapeptide by solid-phase and by classical reactions, and data on hormonal activity which included the corresponding decapeptide-OH or pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH.

Geiger et al. (4) described their synthesis of the decapeptide by classical reactions and compared the properties of their synthetic peptide with active preparations of LH-RH from porcine hypothalami.

Matsuo et al. (5) reported a synthesis of the decapeptide by a solid-phase

*Hypothalamic Hormones XXXVII.

method. Their synthetic product showed the same chemical and biological properties as the natural LH-RH/FSH-RH which they had isolated from porcine hypothalami. Rivaille et al. (6) reported a synthesis of the decapeptide by using a benzylhydrylamine resin in a solid-phase procedure.

Sievertsson et al. (7) detailed two syntheses of the decapeptide, and countercurrent distribution data in the interest of establishing purity, and hormonal activity and potency. Chang et al. (8) described in detail a synthesis of the decapeptide by classical reactions which is particularly advantageous in its versatility for the synthesis of certain analogs. By this synthesis, they also achieved the Lys^8-analog or pGlu-His-Trp-Ser-Tyr-Gly-Leu-Lys-Pro-Gly-NH_2.

We have now synthesized the decapeptide by another new procedure, which has been very effective not only in making available larger amounts of the pure synthetic hormone, but the key steps are very usefully serving for the continuing synthesis of many new structural analogs of LRH.

By this new procedure, pGlu-His-Trp-O-Bzl-Ser-O-Bzl-Tyr-Gly-OBzl (8) was subjected to hydrogenation to obtain pGlu-His-Trp-Ser-Tyr-Gly-OH. This unprotected hexapeptide was then coupled with Leu-Arg-Pro-Gly-NH_2 by the DCI method. The decapeptide, obtained by this procedure, was purified over a CMC column; the yield was about 56% without further development.

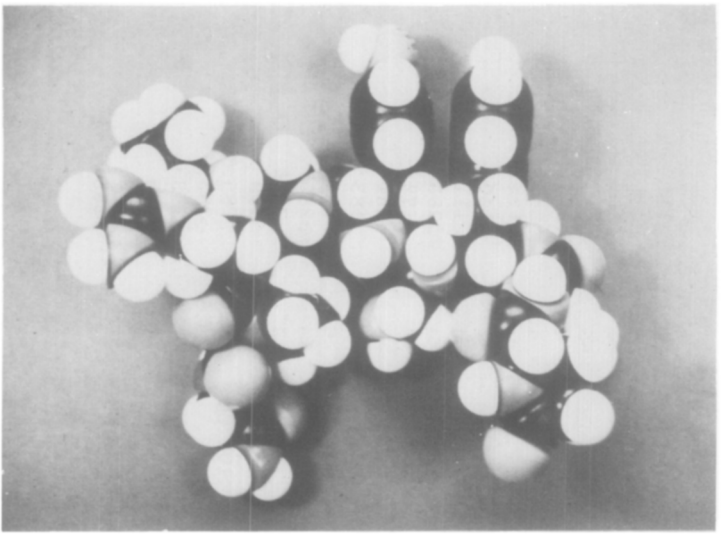
pGlu-His-Trp-Ser-Tyr-Gly-OH was coupled with Leu-His-Pro-Gly-NH_2, Leu-Nva-Pro-Gly-NH_2 and Leu-Pro-Gly-NH_2 by the DCI method to give the following Arg^8-LRH analogs:

His^8-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Leu-His-Pro-Gly-NH_2
Nva^8-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Nva-Pro-Gly-NH_2
Des-Arg^8-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Pro-Gly-NH_2

These analogs are decapeptides, with the exception of Des-Arg^8-LRH which is a nonapeptide. The products were purified on CMC columns in a manner similar to the purification of the decapeptide. The peptides were chromatographically characterized by single spots in one to three systems according to Table I. For this classification, the Des-Arg^8-LRH is included.

The hormonal evaluations in vivo of these new Arg^8-analogs of the luteinizing releasing hormone were performed by using Sprague-Dawley female rats. After ovariectomy and 72 hours before the administration of the peptides, the rats were injected with 50 μg of estradiol benzoate and 25 mg of progesterone according to Ramirez and McCann (9). Under anesthesia, blood samples were taken from the jugular vein and the solutions of the peptides were injected into this vein. The assays of the serum samples for LH were performed in duplicate by the double antibody radioimmuno assay of Niswender et al. (10). The levels of LH are expressed in terms of ng/ml of LER-1240-2-0.60 NIH-LH-SI units/mg.

RESULTS AND DISCUSSION.- A model (Figure I) reveals that the conformation of



the decapeptide could be such that the planar indole and benzenoid moieties of Trp and Tyr could be in relatively parallel positions, because of possible π-π bond interactions. This parallel planarity may bestow some structural specificity for hormonal activity to this molecule. The guanidino moiety of Arg could be relatively extended and exposed. On the basis of these conformational aspects of the decapeptide, and the basicity and the exposure of the guanidino moiety of Arg, the positive charge of Arg could participate in the mechanism of the releasing activity. Such a view of the ionic role of Arg maybe comparable to the view of Sievertsson et al. (11) of the ionic role of His in the thyrotropin releasing hormone (pGlu-His-Pro-NH_2) for function at its receptor site.

These considerations of conformation of the decapeptide as well as an ionic role for Arg in the releasing mechanism of the decapeptide have been a basis for the synthesis of the Arg^8-analogs and their biological evaluation for hormonal activity as described herein.

New data on the hormonal activity of the synthetic decapeptide and related Arg^8-peptides are summarized in Table I.

The synthetic decapeptide as the reference hormone, released LH in the rat assay at 1 ng and showed essentially maximal release at 5 ng. Previously (7), this hormone released LH in our assay at 500 pg. Although significant release of LH by the hormone can be demonstrated at dosage less than 1 ng, dosages of 1-5 ng are useful for comparison of synthetic LRH with analogs.

The decapeptide-OH showed significant release of LH at a dosage up to 5,000-fold that of LRH, and showed maximal release of LH at 40,000-fold level.

TABLE I. ACTIVITY OF SYNTHETIC LUTEINIZING RELEASING HORMONE AND RELATED ARG^8-PEPTIDES

Peptides (a)	tlc Values (b)			Dosage	ng LH/ml. Serum		Dosage	ng LH/ml. Serum	
	R_f^1	R_f^2	R_f^3		Before	After		Before	After
LRH	0.64	-	0.37	1 ng	7.2	216	5 ng	4.2	266
					<4	162		4.0	238
					16.8	168		4.0	>286
					4.0	109		>4	205
LRH (OH)				25 µg	4.4	86	200 µg	<4	>285
					5.8	252		4.6	>285
								4.0	>285
								<4.0	128
Lys^8-LRH	0.65	-	0.31	5 ng	8	11.4	100 ng	<4	140
					6	8		4	111
				25 ng	4.4	41	300 ng	<4	>285
					9.6	95		<4	172
					6	73.6	500 ng	6.2	251
					<4	28.8		6.0	183
				50 ng	<4	16	900 ng	6	>285
					12	68		<4	>285
His^8-LRH	-	0.66	0.51	50 ng	4	12.8	1 µg	<4	30.4
					<4	26.8		<4	118
				200 ng	4	42.2	100 µg	<4.0	>285.6
					<4	67.2		4.6	>285.6
				900 ng	4	157		4	243
					6	200		<4	>285
Nva^8-LRH	0.63	0.76	0.73	300 ng	<4	34	200 µg	4.4	243
					7.6	95.8		4.4	>285
				900 ng	<4	53.4			
					4	63.2			
Des-Arg^8-LRH	0.61	0.68	0.69	500 ng	4	15.6	100 µg	<4.0	>285.6
					<4	4		<4.0	80
				10 µg	<4	92		19	61
					<4	123		32	103

(a) The systematic names of these peptides are in the text.
(b) R_f^1, R_f^2 and R_f^3 (on Silica Gel G) values refers to the systems: n-BuOH:glacial HOAc:EtOAc:H_2O (1:1:1:1); $CHCl_3$:MeOH:conc.NH_4OH (60:45:20); and EtOH:H_2O (7:3), respectively.

Previously (8), we reported data on the hormonal activity of Lys^8-LRH. Additional data for this analog are in Table I, because of the importance of the exchange of the basic Lys^8-moiety for the basic Arg^8-moiety. Lys^8-LRH did not release LH at 5 ng, but significantly did so at 25 ng. Five additional higher dosages of this Lys^8-analog showed increasing release up to maximal release at 300-900 ng.

On the basis of these assays, it appears that a dosage of only up to about 50-fold of Lys^8-LRH in comparison to LRH is required to elicit comparable release of LH. Lys^8-LRH is quite active to release LH, in vivo, and this activity appears to underscore the significance of basicity of the amino acid moiety in the 8-position for potency.

In further support of the concept of the role of a basic amino acid in the 8-position in the decapeptide are the data on His^8-LRH. Although this analog is considerably less active than Lys^8-LRH, it does exhibit high releasing activity. It is estimated that dosage of His^8-LRH which is not too much higher than 200-fold that of LRH would give maximal release.

The Nva^8-LRH is a particularly interesting analog, because it represents all of LRH with the sole exception of the guanidino group. In other words, the guanidino group of LRH is replaced by hydrogen. Dosages of 300-900 ng of Nva^8-LRH (100-300-fold) released significant LH, and dosage up to 40,000-fold revealed maximal release of LH. The LH-releasing activity of $Nval^8$-LRH reveals the multi-structural functions which influence hormonal activity. However, the possible importance of a positive charge in the 8-position, at least for hormonal potency, is not negated. The overall conformational importance of the decapeptide is perhaps revealed by Nva^8-LRH.

The removal of Arg from the 8-position is exemplified in the analog Des-Arg^8-LRH. The combined structural effects of eliminating the basic guanidino group of LRH and reducing its sequence from 10 to 9 amino acids significantly reduced hormonal activity, and apparently more so than did the change from LRH to Nva^8-LRH.

As for both LRH and TRH (pGlu-His-Pro-NH_2), it is unique in medicinal chemistry to study structure-activity relationships where differences in activity of analogs are up to 10,000-fold and greater. This unique study is possible, because of the extraordinary potency of these hypothalamic peptide hormones at pg-ng-levels, in vivo. It may be that structure-activity differences of analogs of up to 100-fold may be better evaluated in terms of classical medicinal chemistry than can differences of up to 10,000-fold and greater. Although the releasing activities of analogs at such enormously increased dosage seem valid, the molecular-site factors which are invoked in the biological function may involve variables which are not participating in the

functions of analogs which are active in the range of up to 100-fold.

ACKNOWLEDGMENT.- Appreciation is expressed to Dr. David Isaksson and Dr. Bertil Åberg, Kabi/Aktiebolaget, Stockholm, and to The Rockefeller Foundation for their respective support of this research.

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