STUDIES ON ANALOGS OF THE LUTEINIZING RELEASING HORMONE TOWARDS ELUCIDATION OF THE RELEASE MECHANISM

by

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SUMMARY

Seven new analogs of the luteinizing releasing hormone (LRH) have been synthesized and assayed towards elucidation of conformation-backbone-sequence-activity relationships and the release mechanism. Proximity between the positive charge of protonated Arg in LRH and a companion negative charge on the receptor site may be important in an ionic reaction which is proposed as part of the release mechanism. This proposal is supported by the low activity of Des-Leu^7-LRH which could have a positive charge too remote for a normal ionic reaction. The pGlu-hexapeptide sequence released LH; the pGlu-heptapeptide sequence was inactive. Four other peptides showed no activity or the uniquely low activities of the second category.

Matsuo et al. (1) proposed that pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 is the luteinizing releasing hormone of the hypothalamus, and that this decapeptide is actually the intrinsic hormone for the release of both LH and FSH.

Several groups of investigators have reported syntheses of this decapeptide. Monahan et al. (2) reported a synthesis by a solid-phase technique. Sievertsson et al. (3) reported synthesis by a combination of solid-phase and classical reactions. Geiger et al. (4) described synthesis by classical reactions and compared their synthetic peptide with active concentrates of LH-RH from porcine hypothalami. Matsuo et al. (5) reported a synthesis by a solid-phase method, and added that the chemical and biological properties of the synthetic product and the natural LH-RH/FSH-RH from porcine hypothalami were identical. Rivaille et al. (6) described a synthesis by the solid-phase method using the benzhydrylamine resin. Folkers (7) and Sievertsson et al. (8) detailed two syntheses of

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the decapeptide, and countercurrent distribution data to establish purity, hormonal activity and potency.

Chang et al. (9) described a synthesis of the luteinizing releasing hormone (LRH) by classical reactions which has versatility for the synthesis of certain analogs. For example, they also synthesized Lys^8-LRH or pGlu-His-Trp-Ser-Tyr-Gly-Leu-Lys-Pro-Gly-NH_2. Chang et al. (10) reported another new synthesis which was very effective to make available larger amounts of the pure synthetic hormone. This synthesis consisted of the coupling of the unprotected hexapeptide with the tetrapeptide, pGlu-His-Trp-Ser-Tyr-Gly-OH and Leu-Arg-Pro-Gly-NH_2, respectively, by the DCI method. This synthesis was also very useful for the obtaining of analogs of LRH with changes of amino acids in the 7 - to 10-positions, and they described His^8-LRH, Nva^8-LRH and Des-Arg^8-LRH

Additional analogs of LRH have now been synthesized not only to elucidate conformation-backbone-sequence-activity relationships, but to seek inhibitors of LRH. It seems that certain knowledge of peptide structure-activity relationships would facilitate the search for inhibitors. Largely for these reasons and using the procedure (10), pGlu-His-Trp-Ser-Tyr-Gly-OH was coupled with Arg-Pro-Gly-NH_2, Pro-Gly-NH_2, and Leu-NH_2 and the following new analogs have been synthesized:

Des-Leu^7-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Arg-Pro-Gly-NH_2
Des-Leu^7-Arg^8-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Pro-Gly-NH_2
Des-Arg^8-Pro^9-Gly^{10}-LRH	or	pGlu-His-Trp-Ser-Tyr-Gly-Leu-NH_2

Des-Leu^7-Arg^8-Pro^9-Gly^{10}-LRH or pGlu-His-Trp-Ser-Tyr-Gly-NH_2, the first six amino acids of the decapeptide in the amide form, was obtained by ammonolysis of pGlu-His-Trp-O-Bzl-Ser-O-Bzl-Tyr-Gly-OBzl (9), and the resulting protected hexapeptide, pGlu-His-Trp-O-Bzl-Ser-O-Bzl-Tyr-Gly-NH_2 was subjected to hydrogenolysis to remove the O-Bzl-groups of Ser and Tyr. pGlu-His-Trp-Ser-Tyr-Gly-OH was obtained by debenzylation of pGlu-His-Trp-O-Bzl-Ser-O-Bzl-Tyr-Gly-O-Bzl. The available Leu-Arg-Pro-Gly-NH_2 (9), the last four amino acids of the decapeptide in amide form, was assayed.

The first reported synthetic peptide to have the activity of the luteinizing releasing hormone was pGlu-Tyr-Arg-Trp-NH_2 according to Chang et al. (11) and Bowers et al. (12). This unique tetrapeptide, which apparently releases only LH and not FSH was "lengthened" by a solid-phase synthesis to the heptapeptide, pGlu-Tyr-Arg-Trp-Gly-His-Leu-NH_2. This heptapeptide has seven of the ten amino acids in LRH, but the backbone sequence of the heptapeptide is very different from the sequence of these amino acids in the decapeptide.

Chemical and bioassay data on these peptides are in Table I. These analogs of the luteinizing releasing hormone were compared with LRH in

TABLE I. ACTIVITY OF SYNTHETIC LUTEINIZING RELEASING HORMONE AND RELATED 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	0.27	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
Des-Leu^7-LRH	0.18	0.15	-	100 ng	5.6	6	1 µg	5.6	101
					4	6.6		6	16.8
							100 µg	5.8	>285.6
								<4.0	193.0
Des-Leu^7-Arg^8-LRH	-	0.62	0.62	100 ng	4	7.8	100 µg	6.0	17.8
					4.4	4.8		4.0	10.6
				1 µg	<4	5	250 µg	8	8
					4.4	4		4	4
Des-Arg^8-Pro^9-Gly^{10}-LRH	0.64	0.66	0.75	100 ng	<4	10	1 µg	5.2	6.8
					6	6		<4	4.8
							100 µg	<4.0	<4.0
								<4.0	6.0
Des-Leu^7-Arg^8-Pro^9-Gly^{10}-LRH	0.57	0.57	0.69	10 µg	20	47	100 µg	<4	245
					4.2	10.6		9	225
					<4	6	200 µg	6	>285
					<4	<4		7	>285
pGlu-His-Trp-Ser-Tyr-Gly-OH	0.53	-	0.66	200 ng	11	9	200 µg	<4	<4
					7	8.5		11	52
Leu-Arg-Pro-Gly-NH_2	0.19	-	0.10	100 ng	5	4	100 µg	6	10
					9	8		4	19
pGlu-Tyr-Arg-Trp-Gly-His-Leu-NH_2				50 µg	<4	196	200 µg	4	206
					<4	73		<4	20

(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.

hormonal assays in vivo using Sprague-Dawley female rats which were ovariectomized. The peptides were administered to the rats 72 hours after they had been injected with 50 μg of estradiol benzoate and 25 mg of progesterone by the guidelines of Ramirez and McCann (13). Under anesthesia, the blood samples for determination of the levels of LH were taken from the jugular vein and preparations of the peptides were injected in this vein. The serum samples were assayed for LH in duplicate by the double antibody radioimmuno assay of Niswender (14). The levels of LH in Table I are expressed in terms of ng/ml of LER-1240-2-0.60 NIH-LH-SI units/mg.

RESULTS AND DISCUSSION.- A model (10) of the decapeptide reveals that the conformation of the molecule could be such that the planar indole and benzenoid moieties of the Trp and Tyr are in relatively parallel positions, because of π-π bond interactions. Such parallel planarity in the conformation may impart some structural specificity for hormonal activity to this molecule. The model also reveals that the guanidino moiety of Arg can be relatively extended and exposed to interaction at the receptor site. Such possible conformational aspects of the decapeptide, and the basicity and exposure of the guanidino moiety indicate that the positive charge of protonated Arg could participate in an ionic mechanism as part of the releasing mechanism. Such an ionic role of Arg in the releasing activity of LRH is in agreement with the view of Sievertsson et al. (15) of an ionic role of His in the thyrotropin releasing hormone (pGlu-His-Pro-NH_2) for function at its receptor site.

Previous peptide-activity studies (10) indicated that a basic amino acid in the 8-position of a decapeptide for the release of LH may be only one of the multi-structural characteristics of both the hormone and its molecular site for the ultimate expression of hormonal activity. These former studies also indicated the overall conformational importance of the decapeptide separate from any aspect of the importance of the basic guanidino group. The hormonal assays of the seven additional peptides in Table I further contribute to an understanding of the peptide structure-activity relationships of LRH.

Des-Leu^7-LRH is LRH without the non-functional leucine moiety. This Des-Leu^7-analog does not change any functional aspect of LRH, but alters only the sequence from 10 to 9 amino acids and alters conformation. Although LH-releasing activity is greatly diminished, there was release at a 200-20,000-fold range of dosage. One interpretation of the positive, but very low activity of this analog, is that the proximity of the positive charge of the protonated guanidino group is so changed in the conformation of the nonapeptide in comparison to its proximity in the decapeptide that the assumed companion negative charge on the receptor site is too remote for the normal ionic mechanism of release. This consideration of proximity calls to mind

the proximity effect as discussed by Koshland and Neet (16) for a role in enzyme action.

Des-Leu^{7}-Arg^{8}-LRH was essentially inactive at dosages up to 50,000-fold that of LRH, a result which is compatible with the concepts.

Des-Arg^{8}-Pro^{9}-Gly^{10}-LRH which consists of the first seven amino acids of the decapeptide in amide form was inactive at a dosage range up to 20,000-fold that of LRH.

Des-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}-LRH which consists of the first six amino acids of the decapeptide in amide form released significant LH at a dosage range 20,000-40,000 that of LRH. It is structurally interesting that the pGlu-hexapeptide sequence is active for release of LH.

pGlu-His-Trp-Ser-Tyr-Gly-OH is apparently inactive up to 40,000-fold dosage in contrast to its amide form which is active.

Leu-Arg-Pro-Gly-NH_2, which consists of the terminal four amino acids of the decapeptide in amide form was essentially inactive at a dosage up to 20,000-fold that of LRH. This negative result on an Arg-section of the decapeptide is notable.

pGlu-Tyr-Arg-Trp-Gly-His-Leu-NH_2, which may be compared with pGlu-Tyr-Arg-Trp-NH_2 (11), appeared to have greater activity for release of LH at a dosage of 50 μg, but the difference is small.

In contemplating the conformation-backbone sequence-activity-relationships of LRH and its analogs, one may have two categories of interpretation to seek an understanding of the obviously complex and overlapping relationships. The first category may be based on those analogs of LRH which show significant release of LH at dosages up to 100-fold that of the hormone itself. The second category may be based on those analogs which show significant release of LH at dosages of about 10,000-fold and higher than the reference dosage levels of the hormone (10). These enormous differences in the range of valid hormonal activity are observable, because of the extraordinary potency of the hypothalamic hormone at pg-ng-levels.

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