DISCOVERY OF A NEW SYNTHETIC TETRAPEPTIDE HAVING LUTEINIZING RELEASING HORMONE (LRH) ACTIVITY

by

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Summary

A new synthetic tetrapeptide, pGlu-Tyr-Arg-Trp-NH_2, has been discovered which has the activity of the luteinizing releasing hormone (LRH) of the hypothalamus, and is, to our knowledge, the first one reported with LRH-activity. Studies indicated the presence of pGlu, Arg, Tyr, and Trp in bovine LRH and Trp in porcine LRH. Of the six possible tetrapeptides, only pGlu-Tyr-Arg-Trp-NH_2, shows LRH activity. This tetrapeptide is less active than LRH, and is not the hormone, but it is relatively potent. A part or all of the sequence of pGlu-Tyr-Arg-Trp-NH_2 may be identical to part(s) of the sequence of LRH.

All available data on LRH indicate it apparently is a decapeptide and not a nonapeptide reported by others.

We have conducted inactivation studies on concentrates of the luteinizing releasing hormone (LRH) from bovine and porcine hypothalami in order to obtain information on the structure of the hormone during the isolation of the hormone (1,2). These studies were performed both with chemical reagents and with enzymes. By using these reagents and enzymes which have a specificity for certain amino acids, evidence for the apparent presence of the moieties of at least four amino acids in the molecule of LRH has been obtained.

These amino acids are pyroglutamic acid, arginine, tryptophan, and tyrosine. It was considered that the four amino acids could be the only ones in LRH as a tetrapeptide or that there could also be present in the hormone other amino acids which are not detectable by inactivation reactions. If LRH is not a tetrapeptide of these four amino acids, then data on the bioassays of the

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sequences of the four amino acids would ultimately contribute to the knowledge of structure-activity relationships for this hypothalamic neurohormone of such great importance for further research in the endocrinology of reproduction and the control of conception. Consequently, the six possible tetrapeptides of pGlu, Arg, Trp, and Tyr have been synthesized and bioassayed in vivo for LRH activity.

This program of syntheses was successful since it lead to the discovery of one sequence of the four amino acids in a synthetic tetrapeptide which shows significant and usable hormonal activity like that of LRH to release luteinizing hormone from the anterior pituitary gland. This hormonally active tetrapeptide is pGlu-Tyr-Arg-Trp-NH_2, and it is a new substance. To our knowledge, no synthetic peptide showing LRH hormonal activity has yet been described in the literature.

The six sequences of the four amino acids are listed in Table I, and their mobilities in tlc with silica gel G in two different solvent systems are defined. All of the tetrapeptides exhibited color reactions with the Pauly, Ehrlich, Sakaguchi, and chlorine-tolidine reagents, but gave no color reaction with the ninhydrin reagent. Acid hydrolysis of pGlu-Tyr-Arg-Trp-NH_2 yielded Glu, Tyr, and Arg while alkaline hydrolysis yielded all four amino acids. These tetrapeptides have been synthesized by classical methods which will be subsequently described. In these syntheses, the amide form of the carboxyl terminus was emphasized for hormonal activity as in the thyrotropin releasing hormone. Basova and Ginodman (3) reported in 1970 that LRH of porcine origin was not inactivated by carboxypeptidases; this observation indicates that a free carboxyl terminus is not present in the hormone. This observation on porcine LRH was later confirmed by Schally et al. (4).

Of the six possible tetrapeptides, hormonal activity was observed only for pGlu-Tyr-Arg-Trp-NH_2. At comparable dose levels of each of the six analogs, a maximum increase of the LH level was found for pGlu-Tyr-Arg-Trp-NH_2 while the other five tetrapeptides did not release LH.

The subsequently synthesized tripeptides, also listed in Table I, pGlu-Arg-Trp-NH_2, pGlu-Tyr-Trp-NH_2, and pGlu-Tyr-Arg-NH_2, with sequences closely related to that of pGlu-Tyr-Arg-Trp-NH_2, show no LRH activity. When evaluated together, the inactivity of the three tripeptides, the inactivation studies (1,2) and the hormonal activity of the synthetic pGlu-Tyr-Arg-Trp-NH_2 are strong evidence for the presence of the four amino acids, pGlu, Arg, Trp, and Tyr, in LRH. The hormonal activity of the synthetic tetrapeptide alone, separate from the inactivation studies, does not necessarily provide evidence for one or more

TABLE I. BIOASSAY DATA ON HORMONAL ACTIVITY OF SYNTHETIC TRI- AND TETRAPEPTIDES

Peptides[a]	mμg LH/ml serum before	after	tlc-Values[b] R_f[c]	R_f[d]
pGlu-Tyr-Arg-Trp-NH_2	3.3	120.0	0.63	0.58
	4.2	>142.0		
pGlu-Tyr-Trp-Arg-NH_2	2.4	2.4	0.74	0.72
	2.0	2.0		
pGlu-Trp-Arg-Tyr-NH_2	3.0	2.2	0.65	0.59
	4.7	4.2		
pGlu-Trp-Tyr-Arg-NH_2	<2.0	<2.0	0.75	0.72
	2.0	2.5		
pGlu-Arg-Tyr-Trp-NH_2	2.0	2.8	0.63	0.51
	4.1	2.5		
pGlu-Arg-Trp-Tyr-NH_2	6.0	6.0	0.64	0.49
	3.4	3.5		
pGlu-Tyr-Arg-NH_2	4.0	4.0	0.64	0.50
	4.0	4.0		
pGlu-Arg-Trp-NH_2	2.0	2.0	0.60	0.45
	<2.0	2.5		
pGlu-Tyr-Trp-NH_2	4.0	4.0	0.56	0.71
	4.0	4.0		

a) All peptides were bioassayed at a dosage of 200 μg.
b) Silica gel G precoated plates were used (commercially available from E. Merck).
c) $HOAc:BuOH:EtOAc:H_2O$ (1:1:1:1).
d) $H_2O:EtOH$ (3:7).
R_f values in systems c and d for His are: c, 0.13; d, 0.03; and for Tyr: c, 0.71; d, 0.57.

or all of these four amino acids in LRH, because of the range of structure-activity relationships of peptide hormones, particularly that of TRH.

The bioassay data in Table I of pGlu-Tyr-Arg-Trp-NH_2 were obtained by the procedure described by Ramirez and McCann (5), and details of the bioassay are described by Bowers et al. (6) together with additional new biological information on the hormonal activities and specificities of synthetic pGlu-Tyr-Arg-Trp-NH_2.

In a following publication by Bowers et al. (6), dose-response data for the LRH-activity of pGlu-Tyr-Arg-Trp-NH_2 are reported. It is clearly evident that this synthetic tetrapeptide is not as biologically potent as natural LRH is expected to be. Although the biological potency of natural LRH of proven purity is not yet known, it is evident that dosages of up to 15 ng or 40 ng would probably be effective in our conduct of the rat assay according to our interpretation of the information reported by Schally et al. (7). Since the dose-response data (6) show that the synthetic pGlu-Tyr-Arg-Trp-NH_2

exhibits only marginal activity in this rat assay at a dosage of 50 μg, it is evident that this tetrapeptide is not natural LRH.

The combined inactivation data on bovine and porcine LRH, and the hormonal activity of the synthetic tetrapeptide even at μg-levels point to the probability that a part or all of the sequence of the four amino acids in this tetrapeptide is identical to part of the sequence of the natural LRH. Since the synthetic tetrapeptide has lower activity than that expected for pure LRH, it is now evident, on this basis also, that the natural LRH has more than four amino acids.

In referring to LRH, Schally et al. (7) stated that "the nonapeptide isolated from porcine hypothalami", and based this assignment on the finding of nine amino acids by acid hydrolysis. Our data on the four amino acids in conjunction with the data of Schally et al. (7) on nine amino acids indicate that LRH is a decapeptide rather than nonapeptide.

We have studied the structure-activity relationships of the thyrotropin releasing hormone (TRH), which is pGlu-His-Pro-NH_2, (8), and found that one amino acid of this tripeptide can be modified with the retention of hormonal activity, but at relatively high dose levels (9,10). However, only inactive compounds were obtained when two modifications of the hormone were made (10). This knowledge on TRH shows that in a small peptide hormone like TRH, minor structural variations can be made without complete loss of hormonal activity. In the case of LRH, which presently appears to be a decapeptide, it has appeared likely to us that structural modifications of LRH can be made particularly in the amino acid units, with varying degree of retention of hormonal activity. Proof of this hypothesis is evident from the hormonal activity of this new tetrapeptide.

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