ON THE FUNCTION OF ARGININE IN LUTEINIZING HORMONE-RELEASING HORMONE

E. HAZUM and M. FRIDKIN
Department of Organic Chemistry

and

R. MEIDAN and Y. KOCH
Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

Many analogs of luteinizing hormone-releasing hormone (LH-RH) have been recently synthesized in order to investigate the significance of the various constituent amino acids in hormonal activity [1]. Assessments of the capacity of analogs to stimulate release of LH and FSH have revealed that arginine in position 8 on the peptide chain is critical for eliciting full activity [2–5]. It was suggested that the basic guanidine side-chain of arginine participates in the binding of LH-RH to its specific pituitary receptor site via an ionic interaction [5] and probably by a hydrogen-bond formation mechanism, as well [3]. It was also postulated, based on fluorescence studies, that the importance of arginine is due to its location in the tertiary structure of the LH-RH molecule itself, that is, in the assembly of the amino acids His^2, Tyr^5, and Arg^8 which exist as a close-knit unit, probably playing an active role in the hormonal action of the peptide [6,7]. The structural function of Arg^8 was also suggested by binding studies of LH-RH and several of its analogs to plasma membranes of the anterior pituitary [8].

To gain further understanding of the function of arginine, we prepared and tested the LH-releasing activity and the cross-reactivity with anti-LH-RH antibodies of two new analogs of LH-RH. The analogs synthesized were (ω-NO_2-Arg^8)–LH-RH, a derivative in which the positive charge of the guanidine group is totally neutralized, and (DHCH–Arg^8)–LH-RH in which the positive character is preserved, while the size of the guanidine is markedly increased. Studies of these analogs support the suggestion as to the structural role of Arg^8 in LH-RH.

Abbreviations: Amino acid derivatives and peptides follow the IUPAC – IUB Commission of Biochemical Nomenclature and symbols as outlined in Eur. J. Biochem. 27, 201–207(1972) and J. Biol. Chem. 250, 3215–3216(1975). DHCH-Arginine N^7,N^8-(1,2-dihydroxycyclohex-1,2-ylene)-L-arginine, DMF *N,N'*-dimethylformamide

2. Materials and methods

Synthetic LH-RH was a generous gift of Ayerst Research Laboratories Saint-Laurent, Canada. 1,2-Cyclohexanedione was purchased from Aldrich Chemical Company, Milwaukee, USA.

2.1. *Synthesis of arginine group (I) analogs of LH-RH*

2.2.1. (ω-NO_2–Arg^8)–LH-RH (II)

p-Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg(NO_2)–Pro–Gly–OH (E. Hazum and M. Fridkin, unpublished) 20 mg, was allowed to couple for 60 min at 0°C in dry DMF (1 ml) with *N*-hydroxy-succinimide (15 mg), using *N,N'*-dicyclohexylcarbodiimide (25 mg) as the coupling agent. Dry ammonia was then bubbled through the solution for 10 min at 0°C, and the crude (ω-NO_2–Arg^8)–LH-RH obtained by evaporation of solvent was purified on a CMC-23 column. Excesses of a starting material were eluted with 0.1 mM NH_4Ac

Table 1
Amino acid analyses and electrophoretic mobilities of LH-RH and its analogs

Peptide		Electrophoretic mobility	Amino acid analyses[b]								
		($R_{LH\text{-}RH}$)[a]	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro
I	LH-RH[c]	1.00	1.02	1.01	n/d	0.98	1.01	2.00	1.02	1.00	0.98
II	[ω-NO_2–Arg^8]	0.57	1.05	0.91	n/d	0.97	1.05	2.02	1.04	–	0.95
III	[DHCH–Arg^8]	0.92	1.04	1.00	n/d	0.95	1.02	1.98	1.05	0.20[d]	0.95

[a] Electrophoresis was performed on Whatman No. 3 paper for 1 h at 60 V/cm in pyridine-acetate buffer (pH 3.5)
[b] Amino acid analyses were performed on a Spinco Beckman Model 120C amino acid analyzer – the peptides assayed were hydrolysed with 6 N HCl containing 4% phenol in evacuated sealed tubes at 110°C for 20 h
[c] Amino acid analysis of the LH-RH used for the preparation of analog III
[d] According to Patthy and Smith [9] there is an 18–20% regeneration of arginine upon acid hydrolysis

n/d = not determined

(pH 6.52), while the product was released using 0.01 M NH_4Ac (pH 6.52).

2.1.2. (DHCH–Arg^8)–LH-RH (III)

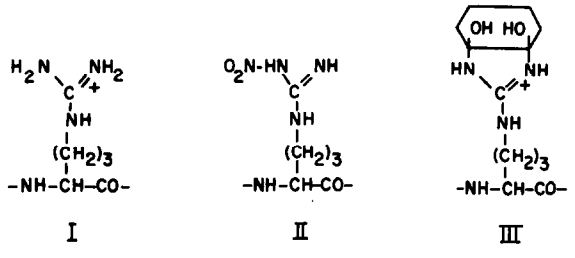
(DHCH–Arg^8)–LH-RH (III) was prepared according to Patthy and Smith [9] by reacting LH-RH (5 mg) with 1,2-cyclohexanedione (20 mg) in sodium borate buffer (0.2 M, pH 9.0, 0.5 ml) under nitrogen in the dark at room-temperature for 24 h. Purification of the crude product was performed by gel-filtration on a Sephadex G-10 column using 1.0 M acetic acid as eluent.

2.2. *Biological assay*

Pituitaries from twelve-day-old female rats were placed in 1 ml of Krebs-Ringer bicarbonate (KRB, pH 7.4) containing glucose (1 mg/ml) and initially incubated for 2 h at 37°C under an atmosphere of 95% O_2:5% CO_2. This medium was then substituted by 1 ml KRB containing LH-RH or an LH-RH analog (1×10^{-9}–5×10^{-7} M), and incubation was continued for 90 min. Aliquots of the medium were then analyzed for LH by radioimmunoassay, using the kit kindly supplied by the National Institute of Arthritis and Metabolism and Digestive Diseases (NIAMDD), rat pituitary hormone program.

3. Results and discussion

Two analogs of LH-RH (I) were prepared in which the arginine residue in position 8 was modified: (ω-NO_2–Arg^8)–LH-RH (II) and (DHCH–Arg^8)–LH-RH (III) (see fig.1). The two peptides were homogeneous when checked by thin-layer chromatography, using various solvent systems, as well as by paper electrophoresis (table 1). Both are positively stained by Pauli reagent for histidine and tyrosine and by Ehrlich reagent for tryptophan, but give negative reaction with Sakaguchi reagent for arginine. Their acid hydrolysates contained the constituent amino acids in ratios close to the theoretical (table 1).



I II III

Fig.1. Structures of arginine residue in LH-RH (I) and of modified arginine residues in (ω-nitro–Arg^8)–LH-RH (II) and in (DHCH–Arg^8)–LH-RH (III).

The LH-releasing activities of II and III were assessed in several dose-levels and found to be 0.8% and 2.4% of LH-RH, respectively. The exceedingly low biological activity of II correlates with its being poorly recognized (<1%) by antibodies specific to LH-RH [10]. The above, coupled with the finding that the fluorescence properties of II are markedly different from those of LH-RH [7], suggests that the neutralization of the positive charge of arginine resulted in a loss of the major structural features of the parent peptide. The somewhat higher activity of III is in line with its being better recognized by anti-LH-RH antibodies (20–25%) and with the fact that its fluorescence properties (E. Hazum and M. Fridkin, unpublished) are not very different from those of LH-RH and are similar to those of (Lys^8)–LH-RH [7]. This latter analog has a substantial LH-releasing activity [2,3] and it highly cross-reacts (80–100%) with anti-LH-RH antibodies [7]. Although some of the essential features of LH-RH were probably preserved in (DHCH–Arg^8)–LH-RH, it is reasonable to assume that masking the positive charge of arginine with the bulky DHCH-group led to changes in the three-dimensional structure of LH-RH.

Hence, it seems plausible that the function of positively charged arginine in position 8 of LH-RH is to stabilize the preferred conformation of the molecule necessary for its normal biological functioning.

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