ON THE IMPORTANCE OF POSITION ONE OF OVULATION INHIBITORS, AS BASED ON STUDIES ON [D-Phe^2, Pro^3, D-Phe^6]-LHRH

by

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Received August 1,1977

SUMMARY

Substitution of cyclopentylcarbonyl-(Cpc) for <Glu^1 in the effective and potent antiovulatory inhibitor, [D-Phe^2, Pro^3, D-Phe^6]-LHRH (I) retained the in vitro potency. We know of no other inhibitor of the luteinizing hormone releasing hormone (LHRH) with a modification at position 1, which is as potent in vitro. This result agrees with the concept of the role of <Glu for agonist activity in a low energy conformer of LHRH, and underscores the importance of position 1. [Cpc^1, D-Phe^2, Pro^3, D-Phe^6]-LHRH did not inhibit ovulation in rats at the same dosage as did I; this result is under study to circumvent. Des-Gly^{10}-[D-Phe^2, Pro^3, D-Phe^6]-LHRH ethylamide and [Glu^1, D-Phe^2, Pro^3, D-Phe^6]-LHRH were significantly less active in vitro than I.

INTRODUCTION

It was common practice in earlier studies of antiovulatory peptides to screen preliminarily LHRH sequences in an in vitro or in vivo assay for their effects on inhibiting the secretion of gonadotropins induced by LHRH. Those analogs which had reasonably high potencies for inhibiting exogenous LHRH were expected to be strong candidates as inhibitors of ovulation.

Bowers et al. (1) have observed some correlation between in vitro and antiovulatory results, but some analogs that were equipotent, in vitro, were not always equipotent in inhibiting ovulation in rats. Nevertheless, all LHRH analogs that inhibited ovulation always inhibited, in vitro, and the most effective inhibitors, in vitro, were most likely to inhibit ovulation.

Yardley et al. (2) reported that des-Gly^{10}-[D-Phe^2, D-Ala^6]-LHRH ethylamide was a more effective inhibitor of LHRH, in vitro, in a monolayer culture system than [D-Phe^2, D-Ala^6]-LHRH (antagonist to agonist ratios of

*PH. 115.

ISSN 0006-291X

1:5 and 20:1, respectively), but at 10-fold the dosage at which [D-Phe^2, D-Ala^6]-LHRH was effective in inhibiting ovulation, the ethylamide analog was inactive.

From conformational energy calculations, Momany (3,4) has indicated that in the lowest energy conformer of LHRH, the N- and C-terminals and the guanidine group of Arg^8 are in proximity. The presence and relative orientation of the ring carbonyl group of <Glu^1 was proposed as important to activate the LHRH receptor (5,6).

Recently, Humphries, et al. (7) reported that [D-Phe^2, Pro^3, D-Phe^6]-LHRH completely inhibited ovulation of rats after a single sc injection of 750 μg; this analog ranks with the presently best known inhibitors.

In view of the likely proximity and biological importance of the N- and C-terminal, we studied the effect of replacing the terminals of the [D-Phe^2, Pro^3, D-Phe^6]-LHRH sequence with certain other funtions.

We describe herein the data on [cyclopentane carboxylic acid^1, (Cpc^1), D-Phe^2, Pro^3, D-Phe^6]-LHRH (I), [Glu^1, D-Phe^2, Pro^3, D-Phe^6]-LHRH (II) and des-Gly^{10}-[D-Phe^2, Pro^3, D-Phe^6]-LHRH ethylamide (III).

EXPERIMENTAL

The peptides were synthesized by automated solid-phase procedures and purified essentially as described for other analogs (7,8). They were checked for purity with the systems R_f^1, 1-BuOH, AcOH, EtOAc, H_2O (1:1:1:1 v/v); R_f^2, EtOAc, py, AcOH, H_2O (5:5:1:3, v/v); and R_f^3, 2-propanol, 1 n AcOH (2:1 v/v). Analytical data are as follows:

[Cpc^1, D-Phe^2, Pro^3, D-Phe^6]-LHRH; $[\alpha]^{24}_D$ -59.61° (c 8.908, MeOH); R_f^1 0.78, R_f^2 0.84, R_f^3 0.76; Amino acid analysis: Phe 2 x 1.07; Pro 2 x 1.08; Ser 0.88; Tyr 0.97; Leu 0.89; Arg 0.99; Gly 0.96.

[Glu^1, D-Phe^2, Pro^3, D-Phe^6]-LHRH, $[\alpha]^{24}_D$ -70.25° (c 10.788, MeOH); R_f^1 0.77, R_f^2 0.80, R_f^3 0.72; Amino acid analysis: Glu 1.04; Phe 2 x 1.08; Pro 2 x 1.03; Ser 0.94; Tyr 0.96; Leu 0.91; Arg 0.94; Gly 0.97.

Des-Gly^{10}-[D-Phe^2, Pro^3, D-Phe^6]-LHRH ethylamide, R_f^1 0.57, R_f^2 0.92, R_f^3 0.74; Amino acid analysis: Glu 1.06; Phe 2 x 1.07; Pro 2 x 1.06; Ser 0.94; Tyr 0.92; Leu 0.88; Arg 0.95.

The peptides were assayed for their activities in vitro and for inhibition of ovulation in rats as described (7,8).

RESULTS AND DISCUSSION

The results of the in vitro assays are in Table 1. As desired and expected, the analogs were essentially devoid of agonist activity, except for a slight release of FSH by the Cpc-analog at 100 μg/ml. It is now generally accepted that effective and potent inhibitors have little or no agonist activity.

Complete inhibition of the release of gonadotropins by 0.6 ng of LHRH occurred with the Cpc-analog at 100 ng/ml. This potency is comparable to that observed for [D-Phe^2, Pro^3, D-Phe^6]-LHRH, in vitro, (7). To our knowledge, no other LHRH inhibitor having modification at position 1 has been reported with such high potency. This result indicates that the <Glu residue in

TABLE I. IN VITRO AGONIST AND ANTAGONIST ACTIVITIES[a]

Anal.	Dose: Peptide μg/ml of medium	Dose: LHRH ng/ml of medium	LH: △ ng/ml of medium	LH: SEM (±)	LH: p	FSH: △ ng/ml of medium	FSH: SEM (±)	FSH: p
I		0.6	145	12		3698	634	
	0.1	0.6	19	6	<0.001	1541	268	~0.01
	1	0.6	11	3	<0.001	441	238	<0.001
			12	4		119	152	
	10		12	5	ns	293	107	ns
	100		22	9	ns	1224	227	<0.01
II		0.6	192	20		2495	161	
	0.1	0.6	123	23	~0.05	2129	407	ns
	1	0.6	89	11	~0.001	1327	116	<0.001
	10	0.6	6	8	<0.001	84	104	<0.001
			5	4		-253	134	
	100		8	2	ns	187	53	<0.02
III		0.6	295	53		3673	463	
	0.1	0.6	252	46	ns	3970	599	ns
	1	0.6	37	32	<0.01	849	280	<0.001
			-25	14		-61	69	
	100		-14	22	ns	165	147	ns

[a]For brevity, not all dosages have been reported.

position 1 of [D-Phe^2, Pro^3, D-Phe^6]-LHRH is not essential for high potency in this in vitro inhibition assay, and underscores new synthetic modifications of position 1.

The Cpc-analog did not show antiovulatory activity at 750 μg, which is the dose at which [D-Phe^2, Pro^3, D-Phe^6]-LHRH was completely effective. Studies in progress may explain this result at this single dosage.

The ethylamide analog (III) inhibited, in vitro, at 1 μg/ml, and was about one-tenth as potent as [D-Phe^2, Pro^3, D-Phe^6]-LHRH. No antiovulatory activity was observed at 375 or 750 μg for III.

The Glu-analog (II) was about one-hundredth less active, in vitro, than [D-Phe^2, Pro^3, D-Phe^6]-LHRH, and did not inhibit ovulation at 750 μg. The substitution of a carboxyl group in position 1 could result in a conformational change which would reduce binding to the receptor (6).

The observation that the Cpc-analog (I) is as potent an inhibitor of LHRH, in vitro, as [D-Phe^2, Pro^3, D-Phe^6]-LHRH, constitutes a new lead in the search for more effective inhibitors of ovulation.

ACKNOWLEDGMENT

Appreciation is expressed to Dr. Marvin Karten and for the support of Contract NIH-NICHD 72-2713 of the National Institutes of Health, and for

Public Health Service Research Grant No. CA-14200-02 from the National Cancer Institute, and for grants from the Rockefeller Foundation and the Robert A. Welch Foundation. The RIA reagents from FSH were distributed by NIAMD, NIH. We are grateful to Dr. G. Niswender, Dr. L. E. Reichert and Dr. Albert Parlow for their RIA preparations and procedures.

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