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AN ANTIOVULATORY DECAPEPTIDE OF HIGHER POTENCY WHICH HAS AN L-AMINO ACID (Ac-Pro) IN POSITION 1*

by

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SUMMARY

Ac-[Pro^1, D-Phe^2, D-Trp^3, D-Trp^6]-LH-RH completely inhibited ovulation in cycling rats at 200μg/rat and is comparable in activity to the corresponding D-<Glu^1-analogue. This Ac-Pro^1-analogue is the most potent antiovulatory peptide yet known having an L-amino acid residue in position 1. This result shows that for the design of potent inhibitors of ovulation, a D-amino acid residue is not essential in position 1. The corresponding Ac-D-Pro^1- and Kic^1-analogues completely inhibited ovulation at 750μg/rat, but not at 200μg/rat, and the Cpc^1-analogue was inactive at these dosages.

INTRODUCTION

Until quite recently, the design of potent inhibitors of ovulation was based on LH-RH sequences structurally modified at positions 2, 3, and 6 (1-5). The most potent ovulation inhibitors based on this strategy were [D-Phe^2, Pro^3, D-Trp^6]-LH-RH (4), [D-Phe^2, Pro^3, D-Phe^6]-LH-RH (5), and [D-Phe^2, D-Trp^3, D-Trp^6]-LH-RH (6), which completely inhibited ovulation in rats at 750μg/rat, s.c.

In studies aimed at achieving more potent inhibitors, we have emphasized the importance of position 1. Analogues of LH-RH having variation in positions

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1, 2, 3, and 6 are exemplified by [chlorambucil[1], Leu[2], Leu[3], D-Ala[6]]-LH-RH (7), the first irreversible inhibitor of LH-RH, in vitro, and [Cpc[1], D-Phe[2], Pro[3], D-Phe[6]]-LH-RH (8), which had the same potency, in vitro, as [D-Phe[2], Pro[3], D-Phe[6]]-LH-RH, but did not inhibit ovulation at 750μg/rat. The Cpc-analogue, however, effectively inhibited the action of LH-RH, in vivo, in adult male rats at a ratio of inhibitor to LH-RH of 100:1 ($p<0.001$) (9).

The antiovulatory potency of 1,2,3,6-tetra-substituted LH-RH analogues is influenced by the structure of the residues in positions 1 and 3. Substitution of the L-<Glu-residue in position 1 of [D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH by a D-<Glu-residue led to increased potency and duration of action (10). However, the incorporation of the same modification into the [D-Phe[2], Pro[3], D-Trp[6]]-LH-RH sequence lowered potency (11).

We now report a group of inhibitors based on the sequence, [Residue[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH, which contain five-membered rings of different characteristics in position 1 and consist of the residues of Ac-Pro, Ac-D-Pro, 2-imidazolidone-4-carboxylic acid (Kic), and cyclopentane carboxylic acid (Cpc). The results are compared with those of the D-<Glu[1]-analogue. Of great significance, is the current finding that in position 1, a D-amino acid residue is not essential for potent antiovulatory activity since the Ac-Pro[1]-analogue is as effective as the D-<Glu[1]-analogue at 200μg/rat.

EXPERIMENTAL

The peptides were synthesized by solid-phase procedures and purified essentially as described for other analogues (5). Purity was checked by TLC on silica gel 60 with the systems (v/v) R_f^1 1-BuOH, AcOH, EtOAc, H_2O (1:1:1:1); R_f^2 2-propanol, 1 N AcOH, (2:1); R_f^3 EtOH, H_2O (7:3); and R_f^4 1-BuOH, pyridine, AcOH, H_2O (30:20:6:24). The presence of Trp was established but not quantitated.

Ac-[Pro[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH: $[\alpha]^{20}$ D -50.84° (c 1.009, MeOH); R_f^1 0.72, R_f^2 0.74, R_f^3 0.81, R_f^4 0.61; Amino acid analysis gave Pro 2 x 0.94, Phe 0.97, Ser 0.92, Tyr 1.02, Leu 1.04, Arg 1.13, Gly 1.03.

Ac-[D-Pro[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH: $[\alpha]^{20}$ D -24.84° (c 0.976, MeOH); R_f^1 0.70, R_f^2 0.71, R_f^3 0.80, R_f^4 0.62; Amino acid analysis gave Pro 2 x 1.03, Phe 1.01, Ser 0.89, Tyr 1.01, Leu 1.03, Arg 1.01, Gly 0.99.

[Kic[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH: $[\alpha]^{20}$ D -50.77° (c 0.993, MeOH); R_f^1 0.72, R_f^2 0.73, R_f^3 0.80, R_f^4 0.60; Amino acid analysis gave Phe 0.89, Ser 0.95, Tyr 1.06, Leu 1.05, Arg 1.04, Pro 0.77, Gly 1.01.

[Cpc[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH: $[\alpha]^{20}$ D -42.6° (c 1.00, MeOH); R_f^1 0.79, R_f^2 0.81, R_f^3 0.84, R_f^4 0.64; Amino acid analysis gave Phe 1.0, Ser 0.9, Tyr 1.0, Leu 1.04, Arg 1.01, Pro 0.90, Gly 0.90.

[D-<Glu[1], D-Phe[2], D-Trp[3], D-Trp[6]]-LH-RH: R_f^1 0.71, R_f^2 0.65, R_f^3 0.65, R_f^4 0.67; Amino acid analysis gave Glu 1.09, Phe 0.90, Ser 1.13, Tyr 0.83, Leu 0.91, Arg 1.01, Pro 1.00, Gly 1.12.

The peptides were assayed for their activities in vitro and for inhibition of ovulation in rats as described (5).

RESULTS AND DISCUSSION

The data from the in vitro assays are in Table 1. As expected, the analogues had essentially no agonist activity at the highest dosages tested.

TABLE 1. ASSAYS, IN VITRO, FOR LH-RH ANTAGONIST AND AGONIST ACTIVITY OF THE [RESIDUE[1], D-PHE[2], D-TRP[3], D-TRP[6]]-LH-RH ANALOGUES[a]

Position 1 Residue	Dose ng/ml of medium Peptide	Dose ng/ml of medium LH-RH	LH Δ ng/ml of medium	LH SEM (±)	LH p value	FSH Δ ng/ml of medium	FSH SEM (±)	FSH p value
Ac-Pro	-	0.6	1003	178	-	5441	664	-
	30	0.6	2	2	<0.001	922	103	<0.001
	100	0.6	-12	10	<0.001	1303	211	<0.001
	1000	0.6	1	11	<0.001	352	204	<0.001
	-	-	52	24	-	-386	183	-
	1000	-	-6	13	ns	-36	235	ns
Ac-D-Pro	-	0.6	1003	178	-	5441	664	-
	30	0.6	41	31	<0.001	396	266	<0.001
	100	0.6	4	10	<0.001	1054	232	<0.001
	1000	0.6	20	13	<0.001	43	81	<0.001
	-	-	52	24	-	-386	183	-
	1000	-	-8	8	ns	17	44	ns
Kic	-	0.6	1014	166	-	3370	198	-
	10	0.6	279	48	~0.001	1869	170	<0.001
	30	0.6	311	51	<0.01	2448	174	<0.01
	100	0.6	99	24	<0.001	984	186	<0.001
	1000	0.6	-15	20	<0.001	353	133	<0.001
	-	-	-33	24	-	-82	88	-
	10,100	-	18	24	ns	52	75	ns
Cpc	-	0.6	1003	178	-	5441	664	-
	3	0.6	787	108	ns	4316	352	ns
	10	0.6	230	25	<0.01	3084	354	<0.02
	30	0.6	105	15	0.001	1304	54	<0.001
	100	0.6	53	21	<0.001	795	77	<0.001
	1000	0.6	8	8	<0.001	150	206	<0.001
	-	-	52	24	-	-386	183	-
	1000	-	11	10	<0.05	11	60	ns
D-<Glu	-	0.6	511	85	-	4534	276	-
	3	0.6	256	48	~0.02	4548	393	ns
	10	0.6	204	6	<0.01	2947	183	<0.001
	30	0.6	59	3	<0.001	1588	290	<0.001
	-	-	25	32	-	206	65	-
	30	-	-25	10	ns	-175	124	0.02

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

All of the analogues decreased the LH and FSH response from 0.6ng/ml of LH-RH by at least 50% at a dosage of 30ng/ml, and equal or greater inhibition was observed at higher dosages.

The rat antiovulatory data are in Table 2. The Ac-Pro[1]-analogue completely inhibited ovulation at 750 and 200μg/rat. This is now the most potent antiovulatory peptide having an L-amino acid at position 1. The D-<Glu[1]-analogue was also completely effective at 200μg/rat. Although the Ac-D-Pro[1]- and Kic[1]-analogues completely inhibited ovulation at 750μg/rat they did not inhibit at 200μg/rat. The Cpc[1]-analogue was inactive at 200 and 750μg/rat.

These results demonstrate that for potent antiovulatory activity a D-amino acid residue is not necessary in position 1 of 1,2,3,6-tetra-substituted

TABLE 2. ANTIOVULATORY ACTIVITY OF THE [RESIDUE^1, D-PHE^2, D-TRP^3, D-TRP^6]-LH-RH ANALOGUES IN RATS

Residue in Position 1	Dose µg/rat sc	No. of rats	No. of rats ovulated	No. of ova per ovulating rat	SEM (±)	% Inhibition of ovulation
Ac-Pro	-	7	7	11.5	1.7	0
	750	6	0	0	0	100
	200	4	0	0	0	100
Ac-D-Pro	-	7	7	11.5	1.7	0
	750	4	0	0	0	100
	200	4	4	6.3	2.3	0
Kic	-	7	7	11.5	1.7	0
	750	4	0	0	0	100
	200	4	4	11.8	0.9	0
Cpc	-	7	7	11.5	1.7	0
	750	4	4	11.3	1.1	0
	200	7	7	12.1	1.6	0
D-<Glu	-	5	5	12.4	0.4	0
	200	7	0	0	0	100

LH-RH analogues. Also, the five-membered cyclic amide ring of the <Glu-residue is not essential.

The structure of an acetylated Pro residue is related to the <Glu-structure in that the cyclic amide -CO- of <Glu has been displaced from a rigid five-membered ring and relocated as a side chain substituent on the ring N of Pro. This eliminates the α-NH group of Residue 1 to yield a tertiary amide in which the -CO- would be expected to have more rotational freedom. The Ac-D-Pro^1-analogue is much less active than the Ac-Pro^1-analogue. This may indicate a configurational preference at position 1 for this particular sequence.

The replacement of the cyclic α-NH-CO of <Glu by a cyclic α-NH-CO-NH- moiety gave the Kic^1-analogue which was as active at 750µg/rat in the antiovulation assay as the corresponding L-<Glu^1-analogue (6). However, replacement by an α-CH_2-CH_2-moiety to give the Cpc^1-analogue suppressed antiovulatory activity. The observations that [Cpc^1, D-Phe^2, D-Trp^3, D-Trp^6]-LH-RH, [Cpc^1, D-Phe^2, Pro^3, D-Phe^6]-LH-RH (8), and [Cpc^1, D-Phe^2, Pro^3, D-Trp^6]-LH-RH (11) have no antiovulatory activity at 750µg/rat may indicate the importance of some polar character in position 1 for antiovulatory activity.

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