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ANTAGONISTS OF LUTEINISING HORMONE-RELEASING HORMONE (LHRH). PROGRESS TOWARDS NON-PEPTIDE LEADS

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Abstract. In an attempt to generate information leading to non-peptide antagonists of LHRH, conformationally restricted analogues of LHRH antagonists have been synthesised. Information on the design, molecular modelling studies and biological activity of several analogues including a bicyclic antagonist c[D-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-MeAla], obtained by forming amide bonds between N- and C-terminal ends and the side chains of Glu and Lys residues, are reported here.

Due to the problems associated with peptides as drugs (e.g. shorter duration of action and lack of oral activity), one of the main objectives of peptide research has been to discover non-peptide ligands capable of acting at peptide receptors.[1] The goal has been achieved in a number of cases (e.g. Substance P, vasopressin, angiotensin II and CCK), but mostly the non-peptide leads have originated from random screening. Subsequent modifications have then led to more potent analogues (see ref. 1 for a recent review). In a few cases only (e.g. CCK), the non-peptide leads were generated by systematic modifications of the starting peptide.[2,3]

In our attempts to generate non-peptide ligands which may act as antagonists of LHRH, we started from potent linear peptide antagonists of LHRH. Conformationally restricted analogues of these linear peptides were then prepared. This approach is likely to provide information on the bioactive conformation of LHRH antagonists. A considerable amount of SAR data is available regarding the importance of the amino acid side chains in the antagonist series of analogues (from our own work and the work of several other groups).[4] Based on the bioactive conformation, it may be possible to arrange the important side chains in a non-peptide structure in such a manner so that the resulting compounds may act as antagonists of LHRH.

Before the start of our work on conformationally restricted antagonists of LHRH, it had been suggested, using empirical energy calculations and molecular dynamics approaches, that LHRH and its analogues had several preferred conformations with a bend around the Gly^6 residue - the most favoured being a modified β-bend involving Tyr^5-Gly^6-Leu^7-Arg^8 residues.[5-7] However, it should be emphasised that the bioactive conformation of the agonist may or may not be relevant to the bioactive conformation of the antagonist.

As a starting point to the conformationally restricted antagonists based on the β-bend hypothesis, it occurred to us that analogues of linear peptide antagonists of LHRH containing a ring between the amino acid residues 4 and 9 or 5 and 8 may be able to adopt the above β-bend conformation more easily and, if such a conformation was relevant for the antagonist activity more potent antagonists of LHRH may emerge. Several examples of 5 to 8 cyclised compounds were synthesised and tested as anti-ovulatory agents in normal cycling rats.[8] Three of the most potent compounds of this series (Table 1, compounds 1, 2 and 3) inhibited ovulation in rats with ED_{50} values of 90, 180 and 540 μg/rat, respectively. In each case, the corresponding linear peptide was at least three- fold less potent than the cyclic peptide, thus indicating that the cyclisation between the side chains of the amino acid residues in positions 5 and 8 may be inducing the bioactive conformation. Based on these results and further modelling studies, two bicyclic LHRH

Table 1. Structures of the monocyclic and bicyclic antagonists of LHRH

Number	Structure
1	Ac-D-Phe(p-Cl)- D-Phe(p-Cl)-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala-NH_2
2	Ac-D-Phe(p-Cl)- D-Phe(p-Cl)-D-Trp-Ser-Asp-D-Arg-Leu-Lys-Pro-D-Ala-NH_2
3	Ac-D-Phe(p-Cl)- D-Phe(p-Cl)-D-Trp-Ser-Asp-D-Arg-Leu-Orn-Pro-D-Ala-NH_2
4	D-Phe(p-Cl)- D-Phe(p-Cl)-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala
5	D-Phe(p-Cl)- D-Phe(p-Cl)-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-MeAla

antagonist analogues (Table 1, compounds 4 and 5) were prepared and tested for ovulation inhibitory activity in normal cycling rats. The results are discussed in this paper.

Synthetic Procedures.

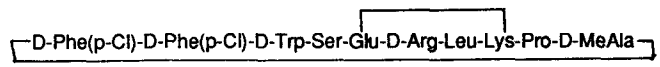
The synthetic details for compounds 1-3 have been reported before.[8] Compounds 4 and 5 were synthesised by the solid phase peptide synthesis method using commercially available Sasrin resin. For the synthesis of compound 4, the fully protected linear peptide Fmoc-D-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser(Bu^t)-Glu(OBu^t)-D-Arg(Mtr)-Leu-Lys(Boc)-Pro-D-Ala was assembled on the resin using N^{α}-Fmoc protected amino acid derivatives with the appropriate side chain protecting groups. After cleaving the N-terminal protecting group (Fmoc), the peptide was detached from the resin by a treatment with 2% trifluoroacetic acid (Tfa) in methylene chloride. The resulting peptide D-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser(Bu^t)-Glu(OBu^t)-D-Arg(Mtr)-Leu-Lys(Boc)-Pro-D-Ala was cyclised by the diphenylphosphoryl azide method described earlier for the synthesis of the monocyclic analogues.[8] The remaining side chain protecting groups (except Mtr) were then cleaved by a treatment with Tfa-water-ethanedithiol-triisopropylsilane (80:5:10:5) (90 min.) and the monocyclic peptide c[Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser -Glu -D-Arg(Mtr)-Leu-Lys -Pro-D-Ala] was cyclised again to give the Arg(Mtr) protected bicyclic peptide 4. Finally the Mtr group was removed by treatment with Tfa-thioanisole (90:10) containing a small amount of tri isopropylsilane for five hours at room temperature and the bicyclic peptide was purified by reverse phase HPLC. The Mtr group has previously been reported to require an extended treatment with Tfa-thioanisole (4 h., 50°C) for its removal from arginine.[9]

The synthesis of compound 5 was attempted initially by a procedure similar to that for compound 4. However, a number of problems were encountered in this synthesis. Firstly, the coupling of Pro to D-MeAla by the diisopropylcarbodiimide method was only achieved in very poor yields and secondly, during the deblocking of the dipeptide Fmoc-Pro-D-MeAla-Resin and further coupling with Fmoc-Lys(Boc), the dipeptide was lost from the resin as a diketopiperazine derivative. The synthesis of this peptide was, therefore, started with Fmoc-Leu-Sasrin Resin and the octapeptide D-MeAla-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser(Bu^t)-Glu(OBu^t)-D-Arg(Mtr)-Leu was assembled by coupling N^{α}–Fmoc protected amino acid derivatives by the HBTU/HOBt/DIPEA method. The coupling of Fmoc-Pro to the octapeptide by the above coupling procedure was not successful. A double coupling step using Bop-Cl[10, 11] and diisopropylethylamine gave the nonapeptide resin. The last amino acid residue Fmoc-Lys(Boc) was coupled by the standard HBTU/HOBt/diisopropylethylamine method. After removal of the Fmoc protecting group, the side chain protected decapeptide Lys(Boc)-Pro-D-MeAla-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser(Bu^t)-Glu(OBu^t)-D-Arg(Mtr)-Leu was released from the resin by a treatment with 2% Tfa in methylene chloride containing ethanedithiol and triisopropylsilane. Following the two cyclisation procedures (as described above for compound 4), the Arg(Mtr) protected bicyclic derivative of compound 5 was obtained. Cleavage of the Mtr group by a treatment with Tfa-ethanedithiol-triisopropylsilane-water gave the bicyclic peptide 5. Like compound 4, bicyclic peptide 5 was also purified by reverse phase HPLC using a gradient of acetonitrile and water containing 0.1% Tfa. Both peptides 4 and 5 were shown to be >98% pure by reverse phase HPLC in several solvent systems. The peptides were characterised by amino acid analysis and FAB mass spectrometry.

Modelling Studies.

Molecular dynamics simulations and energy calculations were performed (as described previously[12]) using a valence force field in which the potential energy of a molecular system is represented as an empirical function of valence degrees of freedom and interatomic distances. Initial studies using a monocyclic peptide Ac-D-Phe-D-Phe-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala-NH_2 (containing an amide bond between the Glu and Lys side chains) showed that the cyclised part of the peptide between residues 5 and 8 did not form a β-turn. This would have been expected from the earlier predictions. In contrast, the peptide adopted a conformation in which it was present in a β-sheet like conformation with a β-turn between residues 3 and 6. In this conformation, the N- and C-terminal ends of the cyclic decapeptide are close to each other and the structure is stabilised by hydrogen bonds typical of a β-sheet conformation as shown in Figure 1. Another conformation with a 5 to 8 β-turn which for long had been presumed to be the bioactive conformation was found to be higher in energy for this LHRH analogue.

In an attempt to reinforce the conformational charecteristics predicted by our modelling studies (a β-bend between residues 3 to 6), we considered the possibility of joining the N- and C-terminal ends of our monocyclic compounds by an amide bond. Molecular modelling studies performed (using a Valence Force Field[13]) by forcibly cyclising two conformations, one with a 3 to 6 type I β-turn and another with a 5 to 8 type II' β-turn, indicate that the former conformation minimises to a lower energy when compared with the later conformation (Table 2). This bicyclic antagonist appears to have similar overall conformational characteristics (Figure 2). As a further restriction, in order to force a folding pattern at the exact place in the sequence as in the previous analogue, we have proposed a model which would constrain a cyclic decapeptide into such a conformation by substitutions on the amide nitrogen of certain residues. In this method (manuscript in preparation) different possible conformations can be excluded and a single predetermined conformation in cyclic peptides selectively forced by appropriate use of N-methyl amino acid residues.

Thus the bicyclic decapeptide 5 (Table 1) 

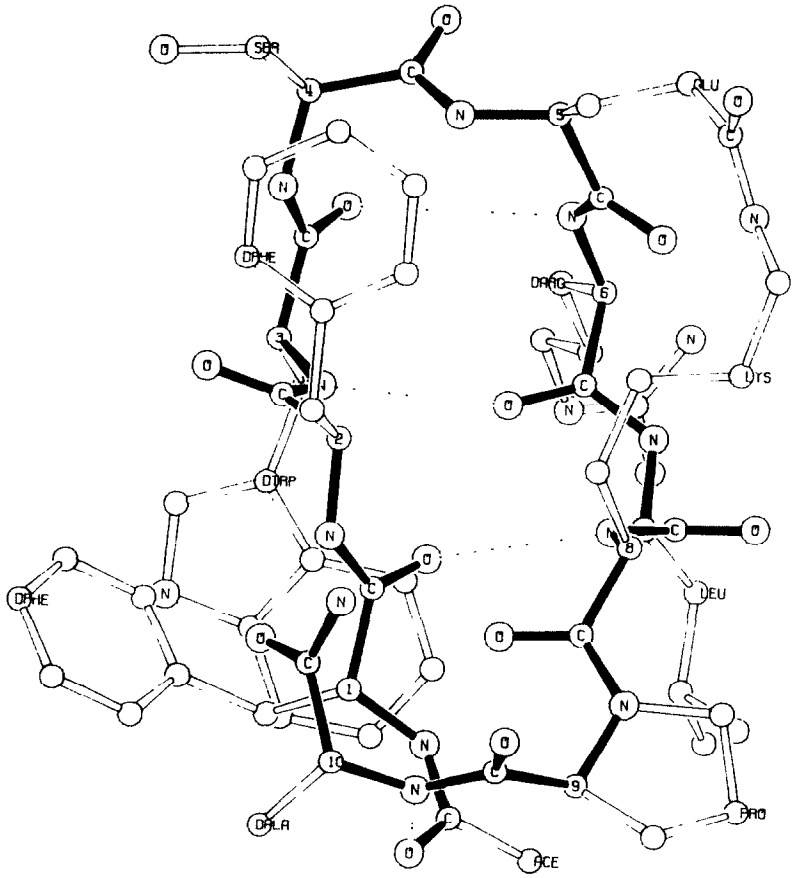


Figure 1. Minimum energy conformation of the LHRH antagonist Ac-D-Phe -D-Phe -D-Trp -Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala-NH_2 containing a type I D-Trp-Ser-Glu-D-Arg β-turn. The backbone is shown with shaded bonds unlike the side chains.

Table 2. Minimum energy conformations of cyclic decapeptide D-Phe(p-Cl)-D-Phe(p-Cl)-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala starting from two different conformations

Conf. No.	D-Phe1		D-Phe2		D-Trp3		Ser4		Glu5		D-Arg6		Leu7		Lys8		Pro9		D-Ala10		Relative Energy*
	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ	
1	65	-73	84	-84	89	-70	-59	-72	-102	47	65	-130	-97	112	-130	161	-67	113	90	38	0.0
2	60	55	80	24	124	-82	-144	107	-79	87	73	-127	-82	-10	-81	122	-74	121	114	-172	13.8

* kcal/mol

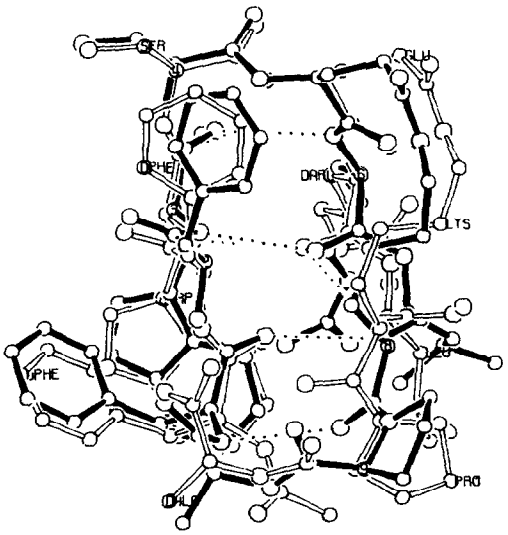


Figure 2. Superposition of D-Phe-D-Phe-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala on the LHRH antagonist of figure 1 shown here with unshaded bonds.

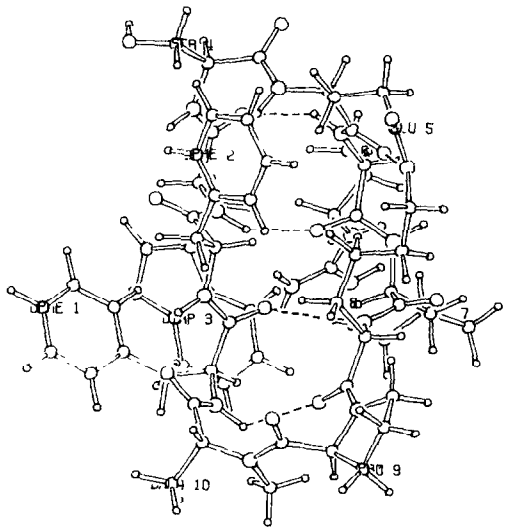


Figure 3. The N-methyl - D-Ala[10] antagonist D-Phe -D-Phe -D-Trp -Ser-Glu-D-Arg-Leu-Lys-Pro-D-MeAla characterised by a Ser-Glu type I β-turn and Pro-N-methyl-D-Ala type II β-turn.

would be expected to have a two β-turn, β-sheet conformation in which the β-turns are across residues Ser^4-Glu^5 and Pro^9-D-MeAla^{10} (Figure 3).

Results and Discussion.

From our earlier work on the synthesis of monocyclic LHRH antagonist analogues and subsequent modelling studies, it appeared that this series of antagonists may be acting at the receptor in a conformation which required a β-bend between residues 3 to 6. To reinforce the low energy conformations containing this β-bend, bicyclic peptides (4 and 5) were synthesised and tested for their antiovulatory activity. The monocyclic peptides were reported previously to be active in this screen and also in a receptor binding assay.[8] As predicted by the model, the D-Ala^{10} analogue (4) was found to be less potent than the D-MeAla^{10} analogue (5). Compound 4 inhibited ovulation (40%) in constant cycling rats at a dose of 2 mg/Kg. In comparison, compound 5 inhibited ovulation (50%) at a dose of 1 mg/Kg. The more potent of the bicyclic peptides (5) (ED_{50} 1 mg/Kg; 200 μg/rat) was similar in potency to the monocyclic peptide 2 and about 5 to 10-fold less potent than the more potent monocyclic peptide 1. Assuming that the loss in potency was not due to adverse pharmacokinetic properties of the bicyclic peptides 4 and 5, it may be concluded that the ring formation between the N- and C-terminal ends of the monocyclic antagonists either leads to some adverse effect on the overall conformation of the peptide or the N-terminal acetyl group present in the monocyclic peptides 1-3 contributes to important binding interactions at the receptor level. The currently available SAR data on the linear antagonist analogues of LHRH does indeed suggest an important role for the N-acetyl group. However, from the point of view of designing non-peptide antagonists, this loss in potency (if associated with the absence of the acetyl group) may not be very significant. If the conformation generated by the bicyclic antagonist 5 is indeed the bioactive conformation, it may be possible to optimise binding interactions by incorporating acetyl or other groups in appropriate positions in a non-peptide skeleton. To make further progress towards the design of non-peptide ligands, our model is being further refined to generate more ideas for the synthesis of more potent conformationally restricted antagonists of LHRH.

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