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A CONFORMATION-PREFERENCE/POTENCY CORRELATION FOR GnRH ANALOGS: NMR EVIDENCE

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Summary: Unlike natural GnRH (which shows a random flexible conformation with a populated reverse turn at residues 3,4), napharelin adopts (in aqueous media containing hexafluoroisopropanol) a non-random conformation in the region of the putative pharmacophore, a type II' β turn at residues 5→8.

Napharelin, [D-β-Naphthylalanyl^6]GnRH, is a potent noncyclic analog of gonadotropin-releasing hormone, GnRH.

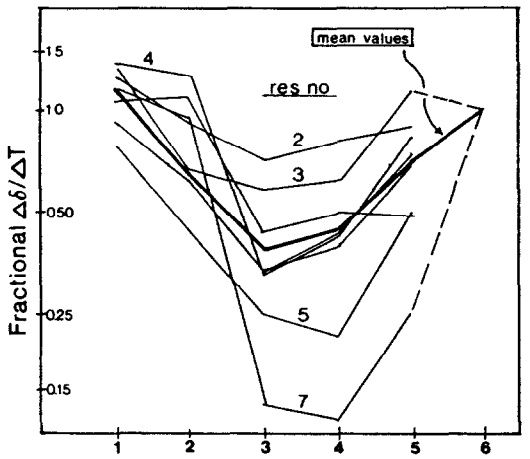
$$\text{pyroGlu} - \text{His} - \text{Trp} - \text{Ser} - \text{Tyr}^5 - \text{D-Nal}^6 - \text{Leu}^7 - \text{Arg}^8 - \text{Pro} - \text{GlyNH}_2$$

It has been shown to suppress the rat estrous cycle with a potency 200 times that of the parent hormone (1). In general, substitution of hydrophobic and/or aromatic D-amino acids for glycine at position six of GnRH has produced more potent (both *in vivo* and *in vitro*) analogs. The increased potency of such analogs has been correlated with increased membrane affinity (2) and also rationalized as a conformational effect (3): the latter, a recognition of the tendency for D-amino acids to promote the adoption of reverse turns (4) in linear peptides. The hydrophobic sidechains may allow the analogs to partition more favorably into biological membranes, a probable step in reaching the receptor (5). It has been postulated (6,7) that bound-GnRH adopts a β-turn configuration at residues 5→8. Structure-activity correlations and NMR studies of cyclic analogs have been viewed as supportive of the hypothesis (8). But there exists no physicochemical evidence for a structuring influence of a D-residue at position 6 in any linear analog.

In solution, noncyclic peptides may sample a large portion of the available conformation space, not necessarily occupying that state associated with a receptor interaction. This appears to be the case for natural GnRH (9). By altering the peptide's medium, it may be possible to effect a change in conformational preference (10), providing a clue as to the importance of specific conformations. In this report we present NMR evidence for a media-induced conformational change for a potent analog which is not seen with the native hormone.

In our study, the solvent systems span a broad range of solvation properties. Those containing DMSO were principally organic media, with denaturing H-bond accepting ability. The solvents containing water had an organic component; either protic hexafluoroisopropanol (HFIP) or polar, aprotic acetonitrile. Aqueous fluoroalcohol media have been reported to minic membrane environments, and numerous instances of peptide structuring similar to that observed in micelles can be cited (11). In each medium and

solvent composition, the resonances were unambiguously assigned using COSY connectivities and sequence-specific NOESY data (12).



-Δδ/ΔT in different media

for media 1→6 see figure legend

residue	1	2	3	4	5	6
H2	6.04	5.35	4.84	4.26	3.77	5.65
W3	5.36	6.14	3.33	3.13	3.91	7.22
S4	6.24	5.19	2.63	2.03	7.90	8.69
Y5	4.72	2.34	1.01	1.18	2.11	3.73
Na16	5.96	4.10	2.36	2.03	5.47	7.52
L7	6.33	1.58	0.70	0.86	6.02	7.34
R8	6.57	4.84	2.79	2.15	—	9.00
G10	6.97	3.40	3.47	3.04	7.64	7.44

Figure 1. Amide NH Shift Temperature Gradients, Media Dependence. The fractional gradient is shown for each solvent. The values in the non-aggregating media (50% aqueous CD_3CN), point **#6**, is used as the standard for each residue. The other media are : **1**, 2:1 benzene/d_6DMSO; **2**, d_6DMSO; **3**, 67% aq. HFIP; **4**, 50% aq. HFIP; and **5**, 20% aq. HFIP.

In **Figure 1**, the relative chemical shift temperature gradients measured for the amide protons are plotted with solvent variation. Actual values are listed in the Table. Clearly, a minimum in $\Delta\delta$ / ΔT is observed in solvents containing higher percentages of HFIP. Superimposed on the general trend are dramatic changes for the leucine and serine NHs, indicating a significant change in environment. Similar effects are not seen for the native hormone (13).

The change in environment is also evident from a comparison of chemical shifts in 500 MHz NMR spectra recorded for amide and alpha protons in 2 : 1 DMSO-Benzene and 67% HFIP (**Figure 2**). Both backbone protons in the leucine residue experienced an unusually large shift toward higher field in HFIP, which is not seen in GnRH. The 7γ proton (at 0.58, *vs* 1.65 ppm in GnRH) is also affected. This can be rationalized by an increasingly populated (in HFPD rich media) conformation which places them above the naphthyl ring plane.

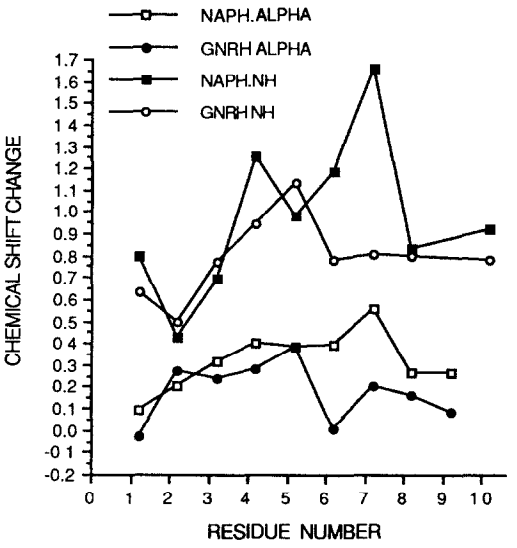
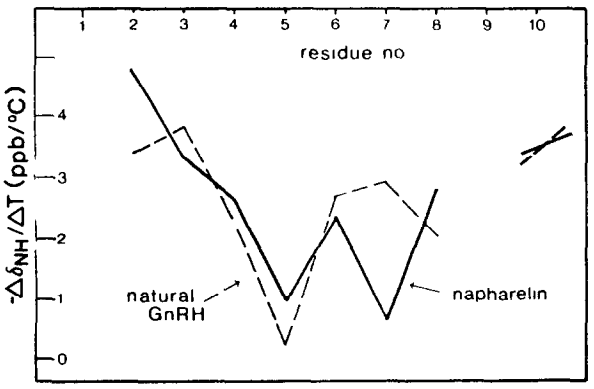


Figure 2. Media Transfer Shifts for GnRH and Napharelin

The profile of $\Delta\delta/\Delta T$ along the peptide chain (below) provides a particularly clear distinction between GnRH and napharelin. The minimum at L7-NH (observed only for napharelin!) could be taken as an indicator of 4→7 turn (4C = 0. . . HN7) (14). We therefore turned to NOESY studies to look for through-space interaction between protons from non-adjacent residues which would be present in a persistent non-extended conformer. These are highlighted in **Figure 3**. The involvement of 6α-H in one such interaction and the high intensity of the 6α / 7NH cross-peak are particularly notable (15). Of the β turns that should be stabilized by the D substitution at position-6 (16), only the type-II′ (5 → 8) and type-II(4 → 7) are concordant with the latter observation. The 6α /8NH interaction requires $\psi_7 \approx 0°$ (and ϕ_7 = -20 ±60°) supporting the (5→8) turn locus. This peak may also have a contribution due to 6α /3ϵ_3: the 8NH and 3ϵ_3 resonances are nearly shift coincident. The alternative attribution also requires persistent medium-range secondary structure.



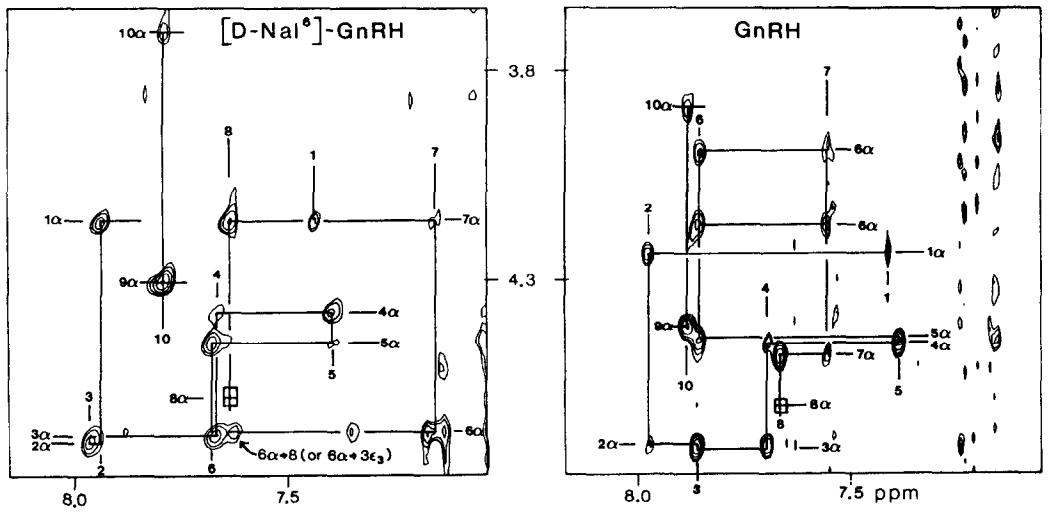


Figure 3. NH/αmethine NOESY segments for 6 mM napharelin and GnRH at 283±2°K in 70% (v/v) aqueous d_1 hexafluoroisopropanol: τ_m = 150 ms (napharelin), 120 ms (GnRH).

To our knowledge the data presented is the first physiochemical evidence for increased stability of the putative pharmacophore region of GnRH upon substitution at residue six. All other recent NMR and CD data for GnRH and non-cyclic analogs has been interpreted as supporting a flexible random coil conformation (9). Detailed modeling and calculations of conformer populations based on the NOESY data sets are in progress. At this stage, we attribute the diminished accessibility of L7-NH to steric interference rather than H-bonding. Parallel studies of another D-Aaa^6 analog lacking an aromatic ring appear essential for a definitive structure refinement.

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