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COMMUNICATIONS

Solute conformation of enkephalin-like pentapeptides in deuterated dimethylsulphoxide

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NMR evidence of the temperature dependence of NH resonances and magnitudes of α–CH–NH couplings is advanced in support of preferred β-bend conformations for five enkephalin-like pentapeptides as solutes in DMSO-d_6. The relevance of these and related conformational studies to the interactions of peptides of the enkephalin class with opioid receptors is questioned.

NMR evidence from temperature dependence studies of NH resonances and the magnitudes of α–CH–NH couplings has been advanced for β-bend species in the cases of leu- and met-enkephalin, as well as the analogue **6** which is terminated by a sulphonic acid residue. Conformations of the former pair are stabilized by a 2–5, and the latter by a 1–4 intramolecular hydrogen bond (Garbay-Jaureguiberry et al 1976, 1977; Bajusz & Casy 1984). The solid-state conformation of **6**, but not that of leu-enkephalin, corresponds with the proposed solute forms (Camerman et al 1983; Stezowski personal communication).

It is necessary to clarify the relevance of such data to the physiological actions of enkephalins if they are to be applied to elucidation of the uptake modes of opioid peptides at the various sub-species of opiate receptor. For this reason NMR studies of enkephalin peptides in deuterated dimethylsuphoxide have been extended to include pairs which differ significantly in potency and selectivity for μ- and δ-receptors, to determine whether such activity variation is reflected in a conformational difference. The pairs examined are the D-Ala^2-D/L-Leu^5 (**1**, **2**) and D-Nle^2-D/L-Nle^5 (**4**, **5**) enkephalin analogues all with the common residues Tyr^1, Gly^3 and Phe^4; D-Ala^2-D-Nle^5-enkephalin (**3**) is included to aid chemical shift assignments.

Tyr-X-Gly-Phe-Y

Peptide	X	Y
1	D-Ala	D-Leu
2	D-Ala	L-Leu
3	D-Ala	D-Nle
4	D-Nle	D-Nle
5	D-Nle	L-Nle
6	D-Nle	L-NleS*

* Analogue of Nle with CO_2H replaced by SO_3H.

Details of the ^{1}H NMR parameters of the α-CH and NH resonances of peptides **1–5** are given in Table 1. Spectra recorded at 400 MHz were well resolved and analysed in a first order manner. Many trivial assignments were possible and others followed by spin decoupling experiments in which the readily identifiable Tyr^1 and Phe^4 β-CH_2 resonances provided the key signals (see footnote *b* of Table 1). Differentiation of the Nle^2 and Nle^5 α-CH resonances of **4** and **5** was possible from data on the D-Ala^2-D-Nle^5 peptide **3**. Changes in the four NH chemical shifts over the temperature range 25–100 °C were also recorded. While it was not always possible to assign all the resonances at elevated temperatures because of signal overlap, it was clear that the highest field resonance (Leu^5 or Nle^5) was the least affected in all cases. The D-Nle^2-D-Nle^5 peptide **4**, a typical example, showed NH resonance movements (ppm) to higher field of: Nle^2 (0·44), Gly^3 (0·37), Phe^4 (0·36), Nle^5 (0·09) at a solute concentration of 6·6 mg ml^{-1} while similar values were recorded at a lower concentration (2·2 mg ml^{-1}). These experiments provide evidence for solute conformations in which the carboxylate terminal NH proton is involved in an intramolecular hydrogen bond with carbonyl oxygen of residue 2 and point to a conformational similarity between all peptides **1–5**. This conclusion is supported by the narrow range of α–CH–NH coupling magnitudes shown for related residues of all the peptides, e.g. Ala^2 7–8 Hz, Phe^4 8–9 Hz. Data of the same kind was reported for spectra of leu- and met-enkephalin (Garbay-Jaureguiberry et al 1976, 1977). Observed α–CH–NH coupling values are consistent with either extended or β-turn peptide conformations within the limits of the approximate ^{3}J vs dihedral angle plots of Cung et al (1974), the latter being favoured by evidence of 2–5 intramolecular hydrogen bonding.

The peptide pairs **1–2** and **4–5** display distinct diastereoisomeric differences in enkephalin-like properties. Thus while **1** and **2** have similar potencies in the guinea-pig ileum assay (**1** 0·31, **2** 0·51, met-enkephalin = 1), the D-Leu^5 isomer **1** is three times more potent than **2** at mouse vas deferens sites (**1** 27·2, **2**

Table 1. First order ^{1}H NMR chemical shifts and coupling constants for α-CH and NH protons of some opioid peptides in DMSO-d_6.[a,b]

Residue	Proton	Peptide 1	2	3	4	5
Tyr^1	α-CH	3·55 t(7)	3·61 t(7)	3·71 t(7)	3·77 t(7)	3·72 t(7)
Ala^2	α-CH	4·19 m, q[c](7·6)	4·15 m, q[c](7)	4·16 m, q[c](7)	—	—
	NH	8·32 bs 7·99[d] d(8)	8·34 d(7)	8·39 d(7)	—	—
Nle^2	α-CH	—	—	—	4·11 m, dd[c](4, 8)	4·12 m
	NH	—	—	—	8·34 d(7)	8·31 d(7)
Gly^3	α-CH	3·60 m	3·65 m	3·61 m, q[c](16)	3·61 m (16, 5·5, 5·5)	3·66 m
	NH	8·56 bs, t at 45° (5)	8·03 t(5·5)	8·24 t(5)	8·27 t(5)	7·98 t(5·5)
Phe^4	α-CH	4·46 m, dd[c] (4·4, 10)	4·41 m, dd[c] (4, 10)	4·5 m, dd[c] (5, 10)	4·5 m (9, 9, 5)	4·45 m (9, 9, 4)
	NH	8·32 bs 7·95[d] (8)	8·16 d(8)	8·17 d(8·5)	8·13 d(9)	8·18 d(8·4)
Leu^5	α-CH	4·02 m	4·04 m, dd[c](4, 9)	—	—	—
	NH	7·61 d(8)	7·89 d(8)	—	—	—
Nle^5	α-CH	—	—	3·95 m, q[c] (5, 8)	3·97 m, dd[c] (4, 8)	3·96 m
	NH	—	—	7·62 d(8)	7·58 (8)	7·85 d(7)

[a] ^{1}H spectra were measured on a Bruker WH-400 NMR spectrometer operating at 400 MHz. Samples (approximately 5 mg) were dissolved in DMSO-d_6 with TMS as reference and examined without degassing, normally at temperatures close to 25 °C and employing the standard conditions of 32K data points and 32 accumulations with digital resolution of 0·328 Hz per point. Chemical shifts are in ppm from TMS, coupling constants or separations (Hz) in parentheses following chemical shifts.

[b] β-CH_2 resonances of Tyr^1 and Phe^4: m within range 2·73–2·8 ppm and 2·91–2·97 ppm for Tyr^1 and Phe^4 respectively; in each case lower field Phe^4 and higher field Tyr^1 dd components were clearly resolved.

[c] After D_2O.

[d] Repeat run at higher temperature.

Abbreviations: d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad signal or two overlapping signals.

9·0) (Kosterlitz et al 1980). A higher mouse vas deferens potency is displayed by the L-Nle^5 isomer of the **4–5** pair (Bajusz personal communication). In view of evidence for the similar solute conformations of these peptides, and indeed for all pentapeptides with Tyr^1 amino and Leu, Nle or Met carboxylate terminal residues, it is improbable that their solute geometry in DMSO-d_6 has any significant bearing upon their pharmacological receptor interactions.

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