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Conformational properties of deltorphin: new features of the δ-opioid receptor

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Deltorphin is an opioid peptide with the sequence H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2, recently isolated from the skin of *Phyllomedusa sauvagei*. Its enormous selectivity towards the δ-opioid receptor and the similarity of the N-terminal part of the sequence with that of dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), a μ selective peptide isolated from the same natural source, prompted a comparative conformational study. A ^{1}H-NMR study in two different solvent systems showed that the conformational preferences of the N-terminal sequences of the two peptides are similar. The different selectivities towards opioid receptors have been interpreted in terms of charge effects. Besides a general trend consistent with the role of the membrane in the preselection of the peptides, the present study demonstrates the crucial role played by charged residues in the interaction inside the receptors.

Opioid; Selectivity; Deltorphin; Dermorphin; NMR; Conformation

1. INTRODUCTION

Selectivity of opiates towards the different receptor sites (κ,μ,δ) is believed to be of key importance for separating analgesy from several unwanted side-effects. The recent discovery of deltorphin [1,2], a new natural δ peptide opioid, may furnish important clues for a better understanding of the differences between δ- and μ-opioid receptors.

Deltorphin is a heptapeptide, H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2, present in the skin of *Phyllomedusa sauvagei*, a South American tree frog. The sequence was first detected [1] in one of the clones that codes the polypeptide precursor of dermorphin, a well known natural μ-opioid peptide [3], characterized by the unusual presence of D-Ala in the second position (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2). The presence of the same processing signals flanking the sequences of both dermorphin and the novel heptapeptide in the precursor polypeptide, suggested [1] that deltorphin could also be produced in vivo. The synthesis of two analogues, containing either L-Met^2 or D-Met^2, proved that also deltorphin contains a D-amino acid in position two, but, in contrast to dermorphin, this new opioid has an activity prevalently δ [2] instead of μ. Indeed, comparison with the synthetic peptides normally used for δ and μ selectivity tests, i.e. DADLE, DPDPE and DAGO, proved [2] that deltorphin is the most powerful and selective natural δ-opioid peptide known to date. The exceptionally high value of δ selectivity may furnish crucial information on the features that im-

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Abbreviations: DADLE, [D-Ala^2,D-Leu^5]enkephalin; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; DAGO, Tyr-D-Ala-Gly-MePhe-$NHCH_2$-CH_2OH; MVD, mouse Vas Deferens; GPI, guinea pig ileum; $DMSO_{d6}$, perdeuterated dimethylsulfoxide; D_2O, deuterium oxide; DQF-COSY, double-quantum filtered correlation spectroscopy; HOHAHA, homonuclear Hartman-Hahn spectroscopy; 1D, one dimensional; 2D, two dimensional; NOESY, nuclear Overhauser effect spectroscopy; NMR, nuclear magnetic resonance. Standard three letter codes are used for amino acid residue identification

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Table 1

Opioid activity of deltorphin, dermorphin and related fragments in guinea pig ileum (GPI), mouse vas deferens (MVD) and radioreceptoral assays expressed as IC_{50} (nM)

Peptides	GPI	MVD	GPI/MVD	[^{3}H]DADLE	[^{3}H]DAGO	[^{3}H]DAGO/[^{3}H]DADLE
Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 [a]	2460	1.09	2.3×10^3	-	-	-
Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 [a]	1.07	23.16	0.05	-	-	-
Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 [b]	3.3	29	0.11	-	-	-
Tyr-D-Met-Phe-Gly-Tyr-Pro-Ser-NH_2 [b]	120	424	0.28	-	-	-
Tyr-D-Ala-Phe-Gly-NH_2 [c]	-	-	-	818.1	36.7	0.04
Tyr-D-Met-Phe-Gly-NH_2 [c]	-	-	-	467.0	9.1	0.02

[a] [2]
[b] [4]
[c] [5]

part δ or μ specificity to opioid peptides, especially in comparison with peptides of the dermorphin family, whose message domain (Tyr-D-Ala-Phe), very similar to that of deltorphin (Tyr-D-Met-Phe), is generally considered responsible for their μ selectivity. Table 1 shows that dermorphin has a relative μ/δ activity (measured as the ratio of the IC_{50} values found in the MVD and GPI tests) ranging from 9 to 20, whereas deltorphin has a relative δ/μ activity (measured as the reciprocal of the ratio used for dermorphin, i.e. as the ratio of the IC_{50} values found in the GPI and MVD tests) of 2.3×10^3.

We have already shown that peptides with Tyr-D-Ala-Phe as their N-terminal sequence can be good μ- and/or δ-opioids, with the only difference that δ agonists have larger hydrophobic side chains in the C-terminal residues [6–8]. Substitution of D-Ala^2 with D-Met^2 cannot possibly be responsible for a selectivity reversal since there are many synthetic [D-Met^2]analogues of dermorphin that possess high μ specificity (table 1). However, before drawing conclusions on the influence (on μ/δ selectivity) of sequential effects, it is essential to investigate the conformational preferences of deltorphin, in particular with respect to dermorphin [6,10,11], since both peptides have considerable flexibility [9].

Here we present a preliminary conformational analysis of deltorphin, based on a proton NMR study in $DMSO_{d6}$ and in the $DMSO_{d6}/H_2O/D_2O$ cryoprotective mixture [12]. The similarity of the conformational preferences of dermorphin and deltorphin allows an interpretation of the μ/δ selectivity of deltorphin on the basis of a specific sequential effect.

2. MATERIALS AND METHODS

Deltorphin was synthesized by classical solution methods and purified by means of silica gel column chromatography, eluted with ethyl acetate/pyridine/acetic acid/water (60:20:6:11) and reverse-phase HPLC on C_{18}, eluted with a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. The final heptapeptide, m.p. 162–164°C, $[\alpha]_D^{26}$ −9.2 (*c* 1.0, methanol), crystallized by methanol/diethyl ether, was isolated as ditrifluoroacetate.

All spectra were run at 400 MHz on a Bruker AM-400 spectrometer equipped with an Aspect 3000 computer and a variable temperature unit. Samples for NMR measurements were 6 mM, both in neat 99.98% $DMSO_{d6}$ (Aldrich, Milwaukee, WI), or in a 80:10:10 (v/v) $DMSO_{d6}/H_2O/D_2O$ cryoprotective mixture. All chemical shifts refer to internal tetramethylsilane. Phase-sensitive DQF-COSY [13], NOESY [14] and HOHAHA [15] spectra were run at 300 K and at 280 K, for the DMSO and the cryoprotective mixture solutions, respectively. NOESY spectra were acquired using a 300 ms mixing time, with 8% random variation in order to minimize scalar and zero quantum contributions. Water suppression has been performed by preir-radiation of the water signal in the gated mode during the recycle and the mixing time. HOHAHA spectra were acquired in the low power transmitter mode; the 90° flip angle in this condition was 70 μs. The total mixing time was 45 ms.

3. RESULTS AND DISCUSSION

Full proton assignments to residue types, in the two solvent systems, were achieved by means of phase-sensitive DQF-COSY and HOHAHA 2D spectroscopy techniques. Sequential and side-chain assignments, based mainly on NOESY experiments, were deemed essential even for a preliminary study, since we wanted to compare the chemical shift of the β-protons of D-Met^2 to that of D-Ala^2 in dermorphin. Accordingly it is necessary to assign unambiguously all side-chain resonances of both D-Met^2 and L-Met^6 residues before drawing conclusions on the conformation

of the peptide. Fig.1 shows the 1D spectrum of deltorphin in $DMSO_{d6}$, with the assignments labeled by standard one-letter codes for amino acid residues.

Table 2 summarizes relevant spectral parameters for the labile backbone protons in the two solvent systems employed; most of the chemical shifts have values consistent with standard literature values [16]. None of the temperature coefficients are close enough to zero to indicate conclusively the presence of stable hydrogen bonds [17], but it may be noted that they are spread over a rather large range. Accordingly, it is possible that the smaller values reflect the contribution of folded conformers in equilibrium with more extended conformations. Fig.2 shows a portion of the phase-sensitive NOESY spectrum of deltorphin in the cryoprotective mixture at 280 K. This spectrum,

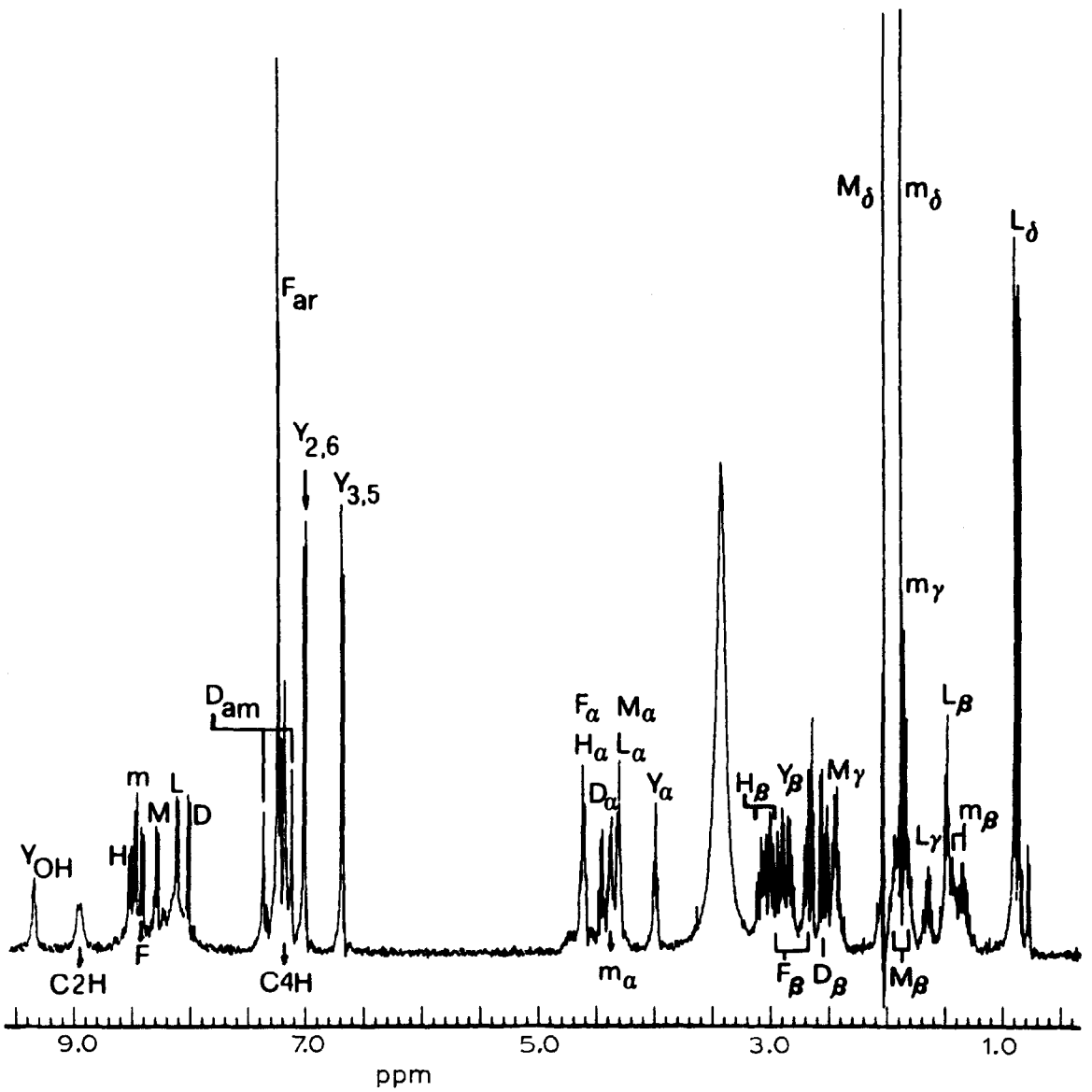

Fig.1. 400 MHz proton spectrum of deltorphin in $DMSO_{d6}$ (3 mg/0.5 ml) at 300 K. All resonances are labeled by the standard one-letter codes for amino acid residues; lower case letter stands for D-residue.

Table 2

Chemical shifts (δ at 295 K) and temperature coefficients ($\Delta\delta/\Delta T$) of amide protons of deltorphin

	DMSO		DMSO/H_2O/D_2O (80:10:10)	
	δ	$\Delta\delta/\Delta T$	δ	$\Delta\delta/\Delta T$
Tyr^1	-	-	-	-
Met^2	8.47	-3.7	8.45	-4.0
Phe^3	8.41	-5.3	8.39	-5.9
His^4	8.52	-7.0	8.44	-7.0
Leu^5	8.11	-4.0	8.21	-4.9
Met^6	8.28	-4.8	8.34	-6.8
Asp^7	8.02	-4.8	8.08	-5.3

with respect to the analogous one in neat $DMSO_{d6}$ at room temperature, is characterized by the presence of a much larger number of NOEs.

All sequential NH-C_αH and many long range intrachain NOEs are visible. These results indicate that the mixture of conformations present in solution is not made up solely of random extended chains.

The chemical shifts of the side-chain protons of D-Met^2 proved diagnostically valuable. In both solvent systems, all side-chain protons of D-Met^2 have unusually high-field chemical shifts, in particular the β-CH_2 protons (1.30–1.37 ppm in DMSO at 300 K). It had already been noticed in dermorphin and its fragments [10] that the chemical shift of the methyl group of D-Ala^2 is unusually low with respect to the standard value for alanine [16] and that the high-field shift is paralleled by a high μ activity. Thus, although some parameters, related to the backbone protons, point to a disordered state, it is possible that most conformations present in solution have a similar arrangement of residues 2 and 3, that represents a local relative energy minimum. It seems significant that H-Tyr-D-Ala-Phe-Gly-NH_2, in the type II' β-turn conformation consistent with a model of μ receptor [6] based on rigid opiates, has the methyl group of D-Ala^2 and the ring of Phe^3 at a short enough distance to induce a large diamagnetic shift in the resonance of the methyl group of D-Ala. Thus, also in the case of deltorphin, it seems reasonable to assume that the relative arrangement of residues 2 and 3 is similar to that present in the β-turn that fits the model receptor we proposed for μ and δ peptides [6].

Our solution study in polar solvents confirms that the main conformational features of μ- and δ-opioids are similar, at least in the N-terminal region. In fact, it can be said that the sequence Tyr^1-D-Xxx^2-Phe^3, long believed to be characteristic of μ-opioids only, is an ideal feature of both μ- and δ-opioid peptides. The cause of the reversal of selectivity in going from the sequence of dermorphin to that of deltorphin must be sought in the properties of residues 4 to 7. We propose that the key factor causing μ to δ reversal is charge in the message domain.

The importance of charges in orienting the specificity of opioid peptides has already been pointed out by Schwyzer [18–20], in the framework of his theory on the catalytic role of membranes for peptide-receptor interactions [21]. In particular, he has proposed that '... selection for δ receptors is reduced by the effective positive charge'. Thus, it is understandable that deamidation of dermorphin causes a decrease in μ activity, whereas amidation of the terminal carboxyl group of enkephalins decreases the δ activity in favor of a slight increase of the μ activity. Also, the negative charge in the C-terminal part of deltorphin (Asp^7) has a role similar to the C-terminal carboxyl group of enkephalins, and is consistent with the general requirements of Schwyzer's theory.

Acritical use of the membrane compartment concept [18] however has a serious drawback: specific receptor requirements may not be given due consideration. Thus, it is indeed very difficult to explain, solely on the basis of the membrane model, why introduction of the positive charge of His in the fourth residue, i.e. at the border of the β-turn required for recognition of μ and δ peptides [6], instead of lowering the δ selectivity, leads to the natural opioid peptide with the highest known δ specificity. These observations strongly favor a specific role of the receptor in the selection of μ and δ agonists, in addition to the catalytic role of the membrane in their pre-selection.

It seems fair to conclude that the picture of the δ receptor that emerges from the present work is similar to that proposed for the μ receptor [6], with an important difference: the ability to tolerate charges outside the region that recognizes the β-turn of the N-terminal region of the peptides. This view receives further support by the recent discovery, in frog skin, of two new deltorphins

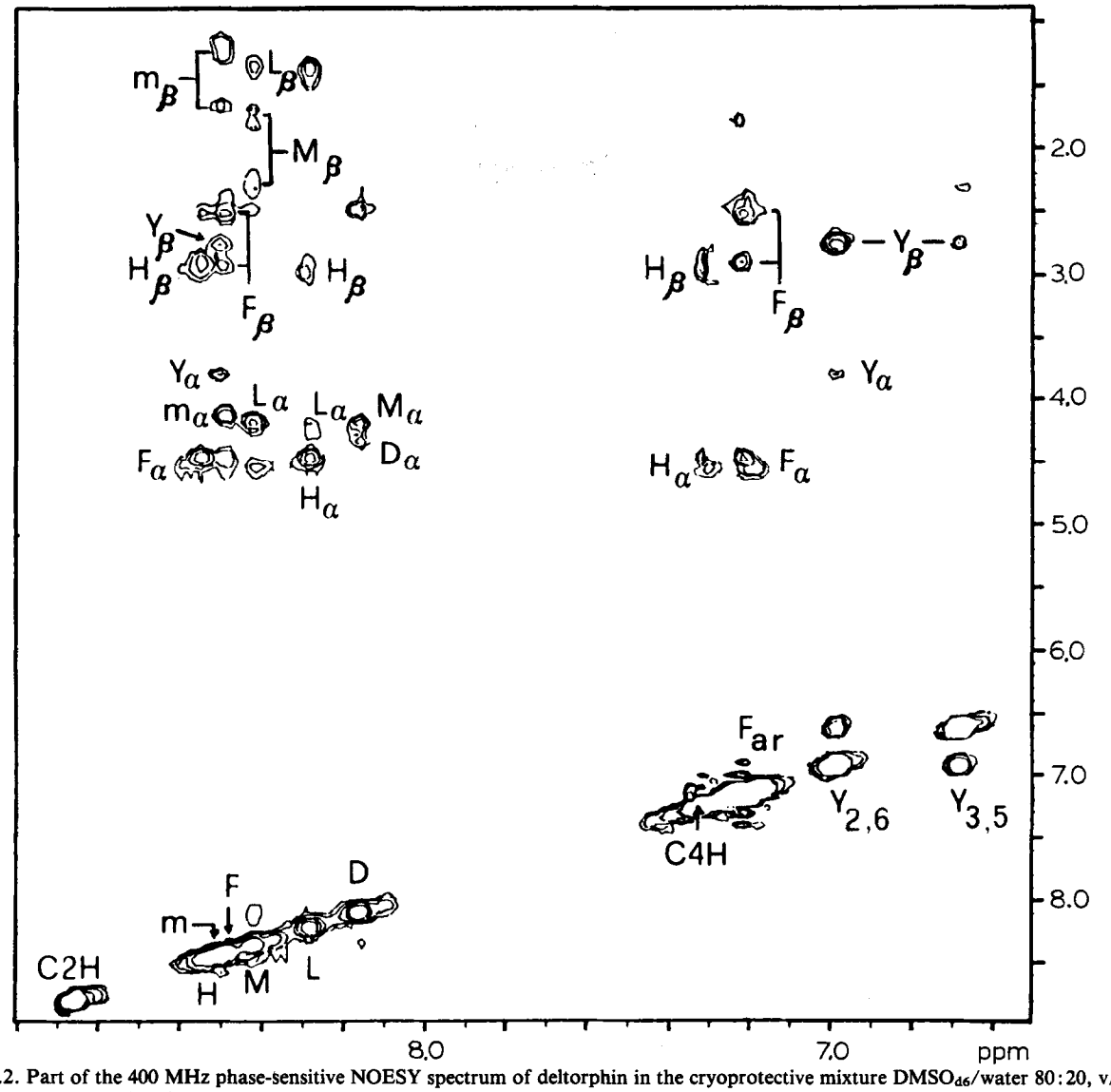

Fig.2. Part of the 400 MHz phase-sensitive NOESY spectrum of deltorphin in the cryoprotective mixture $DMSO_{d6}$/water 80:20, v/v, at 280 K. The mixing time was 300 ms, with 8% random variation. Zero filling of the 2 K × 256 W acquired matrix to 4 K × 1 K has been performed prior to transformation; Gaussian-type windows were applied in both dimensions. Standard one letter codes for amino acid residue labeling have been used, with lower case letters standing for D-residue. This portion of the spectrum shows the dipolar correlations from amide and aromatic protons to α and side chain protons.

(Melchiorri, P., personal communication) that display even higher δ/μ selectivity (deltorphin B: Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2; deltorphin C: Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2). They have a message domain identical to that of dermorphin and bear a negative charge in the fourth residue, showing that the sign of the charge in this position is inmaterial in determining rejection by the μ site.

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