Neurologically active plant compounds and peptide hormones: a chirality connection

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Abstract The most dramatic, but seldom mentioned, difference between alkaloid and peptide opioids is the change of chirality of the α carbon of the tyramine moiety. We propose that the presence of Gly^2 or D-Ala^2 in the two most common message domains compensates this change by allowing the attainment of unusual conformations. A thorough conformational search of Tyr-D-Ala-Phe-NH-CH_3 and of its isomer Tyr-L-Ala-Phe-NH-CH_3 backs this view and establishes a solid link between alkaloid and peptide opioids. This finding supports the notion that morphine, like other neurologically active plant compounds, may bind to endogenous receptors in plants to regulate cell-to-cell signaling systems.

Key words: Alkaloid; Peptide; Signaling system; Bioactive conformation

1. Introduction

Plants produce many complex molecules that are neurologically active; that is, they interact with specific receptors in the nervous system of animals. The prevailing opinion on the role of these substances has assigned them a defensive function against herbivore animals. This view is seriously challenged by the recent discovery of putative glutamate receptors in plants [1]. Although the possibility of a 'plant nervous system' is still farfetched, it seems fair to hypothesize that neurologically active compounds such as caffeine, cocaine and morphine may act as ligands for endogenous receptors in plants to regulate cell-to-cell signaling systems [1]. This finding emphasizes the need for a better understanding of the relationship between neurologically active plant compounds and their animal counterparts.

In this respect, the most interesting case is probably that of opioids, both for their practical relevance as drugs and for the fact that alkaloid opioids, belonging to the family of morphine, were known long before the identification of the corresponding peptide hormones synthesized by animal cells. Since the discovery of enkephalins [2], the availability of a very large number of synthetic analogues of both alkaloid and peptide opioids has favored the collection of a huge body of information on the relationship between primary structure and biological activity (SAR) [3,4] albeit with limited 'cross talk' between the two fields. Indeed, there are several common characteristics between alkaloid and peptide opioids, but also significant differences. Nearly all opioids have part or all of these features: two aromatic rings in a precise spatial disposition, one enol group, and a basic center. In fact, three of these features are embedded in the tyramine moiety that, with the exception of a few synthetic compounds, is common to nearly all opioids. Some of these features may be missing, e.g. the second aromatic ring is not present in morphine, whereas additional features may enhance binding and/or selectivity, e.g. negative charges in δ selective peptides and positive charges in κ selective peptides. Natural peptide opioids have recognition sequences, termed 'message domain' by R. Schwyzer [5] all containing two aromatic residues, Tyr and Phe. These are key residues since their relative spatial position may regulate even receptor selectivity [6]. It is puzzling that the 'bioactive shape' of alkaloids containing two aromatic rings [6], so sensitive to small changes in the relative orientation of the two rings, can be simulated by peptide sequences in which the two aromatic amino acids are separated either by two residues, e.g. the Tyr-Gly-Gly-Phe message domain of enkephalins, or just one, e.g. the Tyr-D-Ala-Phe message domain of dermorphin and deltorphin I [7].

The most dramatic difference is the fact that the chirality of the α carbon of the tyramine moiety in morphine alkaloids is opposite with respect to that of the corresponding carbon atom in L-Tyr, the initial residue in nearly all opioid peptides [8–10]. In spite of its obvious relevance, this inversion of chirality has received little attention in comparisons between alkaloid and peptide opioids. The difference between alkaloids and opioids is even more puzzling if one considers that the whole molecule of morphine is essentially derived from two molecules of L-tyrosine [10]. In addition, cyclization imposes on the χ^1 of tyramine a value of $-90°$ that appears to be essential for opioid activity [9]. The topology requirements imposed by the different chirality of the α carbon of the tyramine moiety and by the rigid scaffold of morphine alkaloids may be achieved in enkephalin, via an induced fit with the receptor, thanks to the extreme flexibility afforded by the two Gly residues [11–13], including the ability of Gly to assume ϕ, ψ values typical of D-amino acids. The role of the two glycines is played by a single D-amino acid in the potent opioid peptides extracted from the skin of South American frogs, which all contain a D-Xaa at the second position of the message domain (Tyr-D-Xaa-Phe) [7,14]. However, the possibility that D-Xaa^2 may confer flexibility comparable to that of Gly is ruled out by the fact that, in addition to D-Ala, bulky residues such as D-Met, D-Leu or D-Val can be found in the second position of deltorphins [7,14]. The presence of D-Xaa^2 in the sequence Tyr-Xaa-Phe may allow the attainment of

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Abbreviations: SAR, structure-activity relationship; SIOM, 7-spiroindanyloxymorphine; Standard IUPAC single- and triple-letter codes for amino acids are used throughout

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(bioactive) conformations that are not readily accessible to Tyr-L-Xaa-Phe and that are consistent with the rigid scaffold of alkaloid opioids. We have performed a thorough conformational search of the tripeptide Tyr-D-Ala-Phe-NH-CH_3 (henceforth called YaF), and of its isomer Tyr-L-Ala-Phe-NH-CH_3 (henceforth called YAF) to check whether energy accessible conformers are consistent with the topological requirements imposed by the chirality of the α carbon of tyramine and with the very rigid constraints imposed to the tyramine moiety by cyclization. The alkaloid chosen for comparison was 7-spiroindanyloxymorphine (SIOM), the first non-peptide δ opioid agonist [15] that similar to all natural peptide opioids, is characterized by the presence of two aromatic rings. In addition, apart from the second aromatic ring, SIOM and morphine have virtually identical common moieties .

2. Methods

Starting structures of H_2N-YAF-NH-CH_3 and H_2N-YaF-NH-CH_3 were generated in fully extended conformations with the InsightII molecular modeling system (Biosym/MS, San Diego, CA, USA). All simulations were performed with the GROMOS program [16] and its standard force field with explicit polar hydrogens. The leap-frog algorithm was used to integrate the equations of motion with a 1 fs time step. Bonds between atoms were kept fixed by the SHAKE procedure [17] with a relative tolerance of 10^{-3}. Non-bonded interactions were handled using a twin-range method [18] with a short and long cut-off radius of 0.8 and 1.0 nm respectively. The non-bonded pair list was updated every 20 fs. A dihedral potential with a force constant of 1000 kJ/mol was applied to enforce when necessary ϕ, ψ angles.

3. Results

In order to find the conformational preferences of these two model peptides we can reasonably limit our search to a conformational space of eight torsion angles, Tyr ψ_1, Ala ϕ_2, ψ_2, Phe ϕ_3 and the χs of Tyr and Phe since the ψ of Phe is uninfluential with respect to the relative orientation of the two aromatic rings. Even restricting the search to staggered conformers of all χs (i.e. g^-, g^+ and t) and using a grid mesh of 30° for the backbone angles, the number of conformations to calculate is discouragingly high. Therefore we resorted to a stepwise approach that could lead to a progressive narrowing of the space to examine.

A preliminary exhaustive search of the conformational space span by the central Ala residues (L-Ala or D-Ala) was performed by restraining (ϕ_2, ψ_2) at a predefined value while minimizing the energy with respect to all other internal coordinates. The need of fulfilling two obvious requirements imposed by comparison with any rigid alkaloid led to the introduction of two further restraints (see Section 2 and Fig. 1 caption). A meaningful comparison of the tripeptides with morphine alkaloids implies a limited range of values for ψ_1, centered around a trans value since, owing to the length of the peptide, values of ψ_1 outside the range −30° to 120° would prevent the ring of Phe from coming even close to the second aromatic ring of SIOM for any combination of the other torsion angles. In addition, the $\chi_1{}^1$ of Tyr must have a g^+ value, consistent with the value found in morphine alkaloids, since it was shown [9] that a change of $\chi_1{}^1$ from −93° (typical

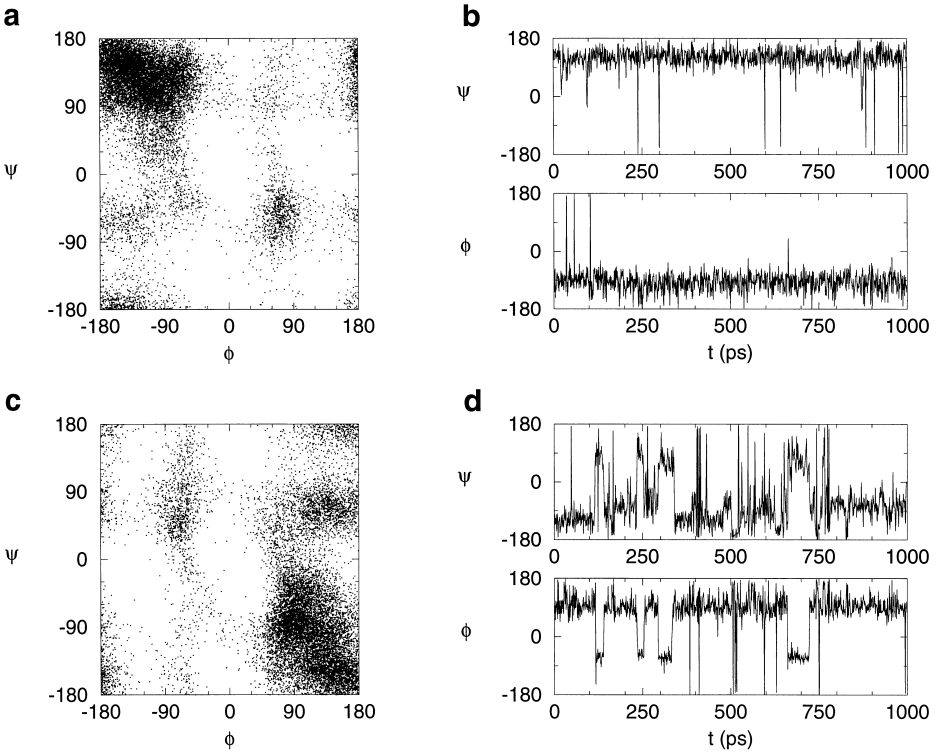

Fig. 1. Conformationally available space for the central residue of the two tripeptides. Calculated Ramachandran plots for YAF and YaF (a and c respectively) with a grid of 30° in ϕ, ψ. Dihedral angles have been restrained to the mapped values, while the ϕ, and χ of Tyr^1 were restricted to values of −90° and −170° respectively. The force constant for all dihedral restraints is of 1000 kJ/mol. After energy minimization, molecular dynamics in vacuo of 100 ps for each grid point has been performed. Displayed points correspond to snapshots sampled every 1 ps. Time evolution of unrestrained simulations of the ϕ, ψ angles for YAF (b) and YaF (d) respectively. Starting configurations have been chosen in the C_7 regions of plots (a) and (c) and energy minimized before starting the unrestrained simulation. Displayed points correspond to snapshots sampled every 1 ps.

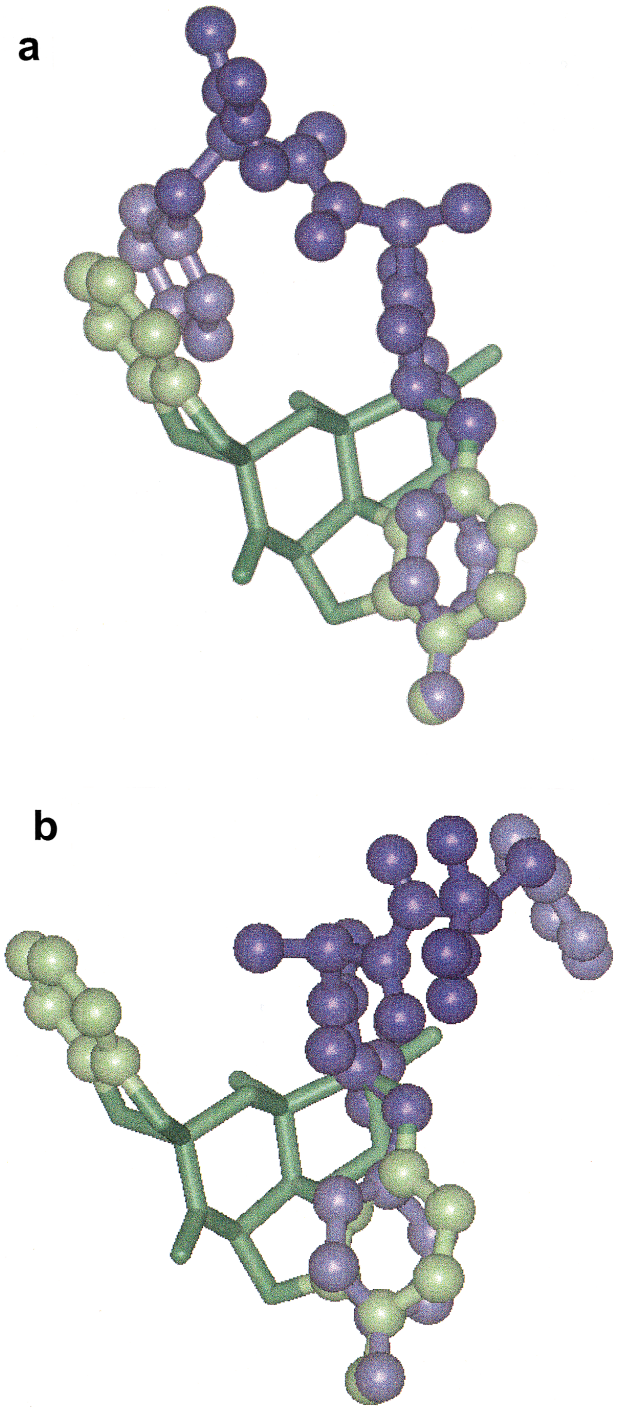

Fig. 2. Comparisons of YaF (a) and YAF (b) with SIOM. The molecular model of SIOM is shown in part as dark green neon (scaffold) and in part as a light green stick and ball representation (aromatic rings). The molecular models of the peptides are shown in part as a dark violet stick and ball (backbone and alanine side chain) and in part as a light violet stick and ball representation (aromatic rings). The overlays were obtained by fitting the basic nitrogen, the α and β carbons, the enol oxygen and the aromatic carbons connected to and para to the oxygen of the corresponding tyramine moieties of the peptide and SIOM.

of morphine and SIOM) to 180° (typical of apomorphine) prevents opioid activity.

Fig. 1 (a, c) shows the ϕ, ψ distributions for the central residue of YAF and YaF respectively, as calculated from Molecular Dynamics simulations. Areas of higher population density are mainly observed in two quasi-antisymmetrical regions of the plots. The centers of gravity of these regions correspond to the well-known C_7 conformations ($C_{7(-/+)}$, $\phi \approx -70°$, $\psi \approx 70°$, and $C_{7(+/-)}$, $\phi \approx 70°$, $\psi \approx -70°$) [19,20]. Antisymmetry can be observed also for other regions of the maps, an indication that the change in chirality of the central residue has a profound effect on the conformational preferences of the whole molecule. Such a result is far from obvious, i.e. it is not a trivial consequence of the different chirality of the central residues, since the two peptides are not enantiomers and all degrees of freedom of the molecule (but ϕ_2, ψ_2) have been left free to evolve during simulations. Fig. 1b,d shows the time evolution of the ϕ, ψ angles for YAF and YaF respectively. Starting configurations have been chosen in the C_7 regions of plots (a) and (c) and energy minimized before starting a long unrestrained simulation. The energy difference between the two C_7 minima is sensibly lower for YaF than for YAF (ΔE=5.2 kJ/mol vs. 19.5 kJ/mol). The conformational landscape is flatter and the number of transitions between the two minima is higher, as can be seen in Fig. 1b,d. All these considerations indicate that YaF is a more flexible and adaptable molecule.

The two highly populated conformers for YaF and YAF were finally compared with SIOM to check whether they are equally consistent with the relative location of the aromatic pharmacophores of a rigid opioid. Fig. 2 shows the overlays of YaF (a) and YAF (b) with SIOM obtained by fitting all heavy atoms of the tyramine moiety, with the exception of *ortho* and *meta* carbons of the aromatic ring. It is easy to see that only the peptide containing D-Ala^2 allows a good superposition of the second aromatic ring whereas the Phe^3 ring of YAF and the corresponding ring of SIOM are spaced in far away regions of space.

4. Discussion

Incorporation of D-amino acids in natural peptides, observed in some bacterial antibiotics, takes place without the participation of messenger RNAs and ribosomes [21]. In the case of all opioid peptides extracted from the skin of south american frogs [7], that are characterized by a Tyr-D-Xaa-Phe message domain, cDNA cloning [22] has shown that a normal codon for the corresponding L-amino acid is present in the prepropeptide. This implies that the D-residues are formed from L-amino acids by a posttranslational reaction [23]. The need for a costly path, such as conversion from L-Ala to D-Ala by a specific isomerase [24], hints that the chirality change may bear important energetic and/or conformational consequences. In fact, it has been hypothesized that the role of the D-Xaa may be to allow the attainment of unusual conformations [24,25]. A possible explanation borne out by the present work, is that the change of chirality of the amino acid in the second position is equivalent to the change of chirality of the α carbon of the tyramine moiety in alkaloids and can influence the conformation of the message domain, in particular the relative arrangement of the aromatic rings of Tyr and Phe. One may wonder why animals, like plants, do not simply change the chirality of the α carbon of the tyramine moiety to achieve the required topological features. However, the stability of the chiral center towards unwanted racemization is assured in morphine, by the rigid alkaloid scaffold. On the

contrary, an α carbon adjacent to a free amino group, e.g. D-Tyr as the first residue of a linear peptide, may be exposed to random racemization more easily than the one of the second residue (embedded in the peptide chain).

Our calculations strongly support the hypothesis of a conformational basis for the use of a D-amino acid residue in the selection of bioactive conformations and establish a solid link between alkaloid and peptide opioids. In turn, this result paves the way to a reconsideration of the role of Gly or D-Xaa residues in the conformation-activity relationship of natural peptides.

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