

# Structural and conformational analogy between cholecystokinin and ergopeptides

RICHARD A. HUGHES AND PETER R. ANDREWS\*

*School of Pharmaceutical Chemistry, 381 Royal Parade, Parkville, Victoria, Australia 3052*

The central nervous system (CNS) peptide cholecystokinin (CCK) and the ergopeptide alkaloids exhibit common pharmacology in the brain, particularly via catecholaminergic systems. We report here structural similarities between CCK and the ergot alkaloids, and the subsequent conformational analysis of the peptide undertaken to establish whether or not a three-dimensional relationship exists between the compounds. Two low-energy conformations of CCK that mimic the ergopeptide ergotamine are identified, one arising from an X-ray crystal structure and the other from a Dreiding model-based, computer-assisted search. The pharmacological, structural and conformational observations strongly support the hypothesis that CCK and the ergopeptides share common sites of action in the CNS.

Cholecystokinin (CCK) is a 33-amino acid peptide initially isolated from the gastrointestinal tract (Mutt & Jorpes 1963), but since found in the mammalian central nervous system (CNS) (Dockray 1976). The carboxy (C-) terminal octa- (CCK-8) and tetra- (CCK-4) peptides appear to be the predominant CNS forms (Rehfeld 1978). Much evidence exists to support a possible role for CCK as a neurotransmitter or neuromodulator, including the identification of specific CCK receptors (Innis & Snyder 1980). Its potential function as a satiety signal, however, appears to involve noradrenergic and/or dopaminergic systems (Yalow et al 1983; Myers & McCaleb 1981; Tsai et al 1984). Indeed, CCK has been demonstrated to interact with catecholamines in a number of studies (Hökfelt et al 1980; Fuxe et al 1980; Fekete et al 1981; Kovács et al 1981; Meyer & Krauss 1983). The ergopeptides, those ergot alkaloids possessing a peptide moiety substituted on the ergoline ring structure (Rall & Schleifer 1980), likewise show affinity for a range of neurotransmitter receptors (Closse et al 1983), including those pertaining to catecholamines. Ergotamine (EA), for example, is an  $\alpha_1$ -receptor antagonist used in the treatment of migraine (Bowman & Rand 1980), while the  $D_2$ -receptor agonist, bromocriptine, is used successfully in the treatment of Parkinson's disease, acromegaly and hyperprolactinaemia (for review see Thorner et al 1980). Similarly, CCK and gastrin, which shares its C-terminal pentapeptide with CCK, exert effects on growth hormone and prolactin control (Nair et al 1984; Vijayan et al

1978). The relationship between CCK, the ergopeptides and catecholamines is thus most intimate.

A possible reason behind the extensive common pharmacology of CCK and the ergopeptides may be found in the striking structural similarities that exist between the two groups of compounds (Fig. 1). Both molecules possess an indole system, a phenyl ring, and a potentially positively charged nitrogen atom. Furthermore, the peptidic backbone contained within EA corresponds almost one-to-one with CCK-4. However, while this indicates that the two molecules are near-perfect topological analogues, it does not establish whether or not they could share common binding sites at a receptor. To do this, peptide and alkaloid would also have to be topographically related, that is, capable of adopting a common conformation.

The conformation of EA has been studied (Pierri et al 1982), with two conformations of EA proposed as potentially biologically active. For CCK, a number of conformational studies have been reported using proton nuclear magnetic resonance spectroscopy (Feeney et al 1972; Bleich et al 1976; Durieux et al 1983), circular dichroism (Pham Van Chuong et al 1979; Penke et al 1983), theoretical calculations (Kier & George 1972; Yamada et al 1976; Abillon et al 1981), and X-ray crystallography (Cruse et al 1980). Favoured conformations range from extended to folded forms, indicating the flexible nature of small peptides such as CCK. The thorough investigation of the topographical analogies between EA and CCK-4 would require a grid search over the entire conformational space available to both molecules producing, ultimately, a set of conformationally

\* Correspondence.

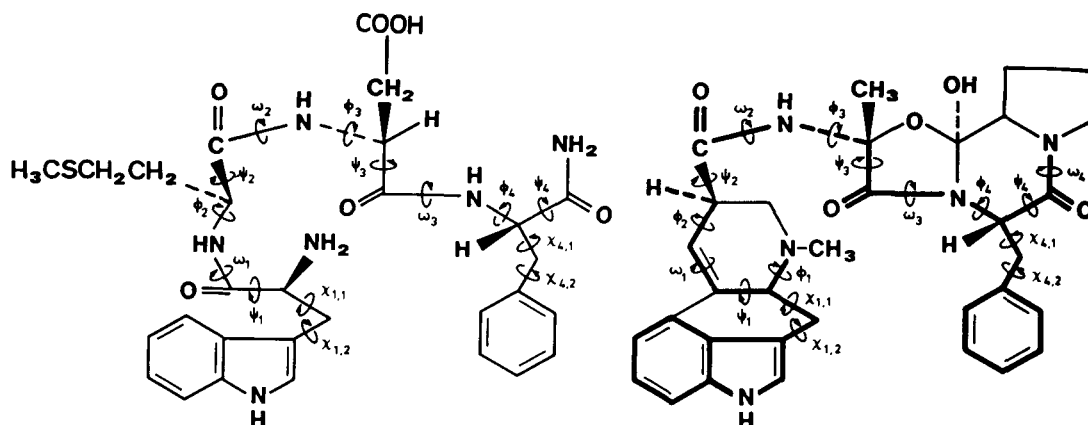


FIG. 1. Cholecystikinin tetrapeptide amide (CCK-4, left) and ergotamine (EA, right). CCK-4 is drawn in such a manner as to highlight the structural similarities between it and EA. The bold portion of EA shows the common aromatic moieties of the two compounds and the peptidic backbone embedded within the ergot structure. In viewing EA in this manner as a conformationally restricted CCK-4 analogue, seven backbone torsion angles ( $\phi_i$  and  $\psi_i$ ) and four sidechain torsion angles ( $\chi_{i,j}$ ) determine the conformation of the peptide. (The torsion angle about the peptide bonds,  $\omega$ , is taken to be  $180^\circ$ , that is, planar, trans amide bonds.)

common models if, indeed, any existed. Now, while such an exhaustive approach would be feasible for studying the ergots, whose conformational freedom is greatly inhibited by their extensive ring systems, the inherent flexibility of peptides makes a grid-searching technique out of the question. Taking the desirable  $10^\circ$  steps about each of the 11 bonds potentially free to rotate would entail sifting through some  $36^{11}$  conformations, while even a rather coarse  $30^\circ$  step size would generate  $12^{11}$  conformations.

Several means of solving this conformational problem other than via a comprehensive, but unrealistic, grid search could be considered. The simplest would be to assume that the crystal structure is representative of the biologically active conformation, and hence use this as a starting point for a limited search. Alternatively, one could propose that CCK-4 adopts a standard, low-energy peptide conformation at its receptor, and then see how this structure relates to EA. A third option would be somehow to sample the conformational space of CCK-4, paying attention to the region in which the peptide might resemble the alkaloid. In fact, all three approaches were used.

In the first approach, the two CCK-4 crystal structures available were superimposed on EA in a potentially biologically active conformation, namely an extended,  $\beta$ -methyl, flap-up and protonated form (Pierri et al 1982), using the computer graphics package MORPHEUS (Andrews & Lloyd 1982). The non-bonded interactions of the methionine (Met) and aspartic acid (Asp) sidechains of CCK-4

were minimized, any apparent improvements to fit and/or energy made manually, and an iterative gradient search method employed to maximize the extent of fit between CCK-4 and EA, whilst minimizing the non-bonded atom interactions of the peptide.

The superimposition was performed using the extended molecule approach (Lloyd & Andrews 1986), which caters for the fact that atoms in two molecules need not occupy exactly the same position in space in order to bind to a common receptor group. For this purpose, the molecules to be superimposed are extended by the addition of receptor guidepoints to their key features. In the case at hand, receptor guidepoints were attached 3.5 Å above and below each aromatic ring system, to represent the possible location of a receptor for either a phenyl or indole group, and 2.8 Å from the protonated nitrogen of each molecule, the likely position of a hydrogen bond acceptor. The guidepoints themselves were then superimposed, rather than actual atoms, and during the gradient search procedure it was the guidepoint deviations that were minimized and finally used to express the extent of fit of CCK-4 and EA.

A  $\beta$ -pleated sheet was used as a starting conformation in the second approach. This standard secondary peptide structure would be expected to be of low energy, free from crystal packing forces possibly present in the X-ray structure used in the first approach, and hence potentially a biologically active form of CCK-4. It was also used to provide an energy baseline against which peptide conformations devel-

oped in approach three could be assessed. The conformational similarity of the  $\beta$ -sheet with EA was determined using the techniques described above.

The third approach made an attempt to sample the vast conformational space available to CCK-4, using Dreiding models as a starting point. As the only peptide conformations of interest were those which most resembled EA, a method of generating such conformations was sought. This was accomplished using Dreiding molecular models to fit CCK-4 to EA. Of the several hundred peptide conformations considered, the torsion angles of 30 conformations that looked sterically acceptable were read, and the resultant pairs of  $\phi$  and  $\psi$  angles obtained for each residue were plotted on a standard Ramachandran diagram for L-alanine (Ramachandran et al 1963). These diagrams were then used to direct further modelling, the goal being to produce CCK-4 conformations with plots of  $\phi$  against  $\psi$  that fell within acceptable regions on the Ramachandran diagram, and thus likely to be energetically accessible to the molecule, whilst still satisfying the constraint of conformational similarity with EA. Sixteen conformations passed the Ramachandran plot test. Using MORPHEUS, the Met and Asp sidechain inter-

actions were optimized and the non-bonded interaction energies of each of the 16 models measured. Those with an energy greater than 40 kcal mol<sup>-1</sup> were discarded. The gradient-search technique described above was then used, leaving eight conformations with a root-mean-square (RMS) deviation of receptor guidepoints of less than 1.6 Å and an energy of less than 10 kcal mol<sup>-1</sup>. The percentage molecular overlap volume of each of these models with EA was determined by the program OVALAP (Hughes unpublished software), with just two conformations having greater than 40% overlap. As a final discriminator, the percentage overlap volume of the peptide backbones of CCK-4 and EA was calculated. Only one peptide conformation managed to pass unscathed through the series of filters. Table 1 outlines the major stages of the search procedure, while results from the three approaches are summarized in Table 2.

Of the conformations arising from the Dreiding model-based search, model IV is the best, with a van der Waals energy some 3.4 kcal mol<sup>-1</sup> below that of the  $\beta$ -sheet conformation. However, although the guidepoint fit of IV with EA is good, it shows poor overlap and was therefore discarded at this stage. Indeed, there is no common volume occupied by the respective peptide backbones. Model V passed all tests except the backbone overlap, showing only 18% overlap with the EA backbone. It was thus difficult to imagine visually the two molecules interacting with a common receptor if V were to be considered the biologically active conformation. This left VI as the only Dreiding-derived model to pass all tests, with an energy 1.1 kcal mol<sup>-1</sup> below the  $\beta$ -sheet, the best RMS deviation (1.1 Å) and very good overall and backbone overlap.

Table 1. Outline of search procedure used to generate CCK-4 conformations that most resemble EA, and the series of filters used to eliminate all but the best of these.

Selection criteria	Tested	Passed
Dreiding model match	>300	30
Ramachandran plot	30	16
Energy cut-off <40 kcal mol <sup>-1</sup>	16	12
RMS cut-off <1.6 Å/energy cut-off <10 kcal mol <sup>-1</sup>	12	8
% Overlap cut-off >40%	8	2
% Peptide backbone overlap cut-off >40%	2	1

Table 2. Torsion angles for the crystal structures (I, II), the  $\beta$ -pleated sheet (III) and three conformations arising from the Dreiding model-based search procedure (IV, V and VI), both before and after the gradient search. Included are the van der Waals-type non-bonded energies for each conformation relative to the unoptimized  $\beta$ -pleated sheet, and the RMS guidepoint deviations, the percentage overlap volume, and the peptide backbone percentage overlap with EA.

Model		$\Phi_1$	$\chi_{1.1}$	$\chi_{1.2}$	$\Psi_1$	$\Phi_2$	$\Psi_2$	$\Phi_3$	$\Psi_3$	$\Phi_4$	$\chi_{A.1}$	$\chi_{A.2}$	Energy (kcal mol <sup>-1</sup> )	RMS (Å)	% Overlap	% Peptide backbone overlap
Crystal structure I	B	180	-66	-84	157	-133	164	-151	115	-85	176	-90	5.0	4.6		
	A	160	-133	-101	143	-118	164	-121	103	-75	-160	-89	8.8	3.4	53	41
Crystal structure II	B	-56	-61	-83	139	-145	158	-144	126	-101	-65	83	6.1	2.4		
	A	-59	-113	-96	145	-132	158	-132	117	-100	-56	83	6.9	1.2	55	44
$\beta$ -Pleated sheet III	B	70	-70	-80	113	-119	113	-119	113	-119	-50	-70	0	2.7		
	A	58	-85	-79	123	-103	116	-109	127	-119	-57	-94	-0.5	1.7	52	39
Dreiding derived IV	B	60	180	-120	120	-60	140	-150	-50	-110	-80	60	-0.5	1.4		
	A	58	180	-118	120	-71	139	-150	-40	-106	-90	60	-3.4	1.4	35	0
Dreiding derived V	B	170	-150	150	60	-150	160	-90	90	-150	60	120	7.4	2.2		
	A	-174	-158	125	70	-151	160	-83	102	-151	60	99	-0.9	1.5	51	18
Dreiding derived VI	B	0	-70	-70	70	-150	-50	-90	150	30	-40	-100	14.3	1.9		
	A	-1	-72	75	-77	-155	-57	-80	150	61	-42	-58	-1.1	1.1	51	45

B = before, A = after.

Crystal structure I bears little conformational resemblance to EA and, as a result, would have been discarded from the search on RMS grounds. Similarly, optimized  $\beta$ -pleated sheet III would have failed to make the grade due to an unacceptable RMS guidepoint deviation (1.7 Å).

Although it was not subject to the same criteria as IV, V and VI, the crystal structure II would have managed to pass all the tests of the search procedure, although its final energy is 8.0 kcal mol<sup>-1</sup> greater than that of its competitor, VI. The fact that II was not encountered during the Dreiding model search reminds one that the latter procedure, although useful, was not exhaustive.

Fig. 2 shows stereo pictures of II and VI superimposed on EA. As delineated in Table 2, II and VI show similar fit and overlap characteristics. This similarity is also apparent to some extent visually, as both peptide conformations show similar sidedness to EA. Interaction with a common receptor, in either case would be feasible. Both models also share a second, albeit different, potential hydrophilic binding group in common with EA, in addition to the charged nitrogen atom used to locate a receptor point in the fitting procedure. Thus in II, the C-terminal carboxyl group is suitably located near

the amide oxygen adjacent to the proline moiety of EA, while in VI, the C-terminal amide group is orientated such that it could share a receptor binding group with the pyruvyl-derived hydroxyl group of EA.

As far as the peptide backbones of II and VI are concerned, although the overlap volume with EA is identical quantitatively, visually it is not. For the greater portion of the backbone, VI appears to give a tighter fit with EA. The good overlap figure obtained with II is due to the better overlap of the C-terminal portion of the peptide with EA. The different peptide backbone orientations of the two conformations invoke different Met and Asp sidechain positions. II has these sidechains located on opposite sides of the backbone, taking the Asp sidechain to a position where it is probably pointing away from, and therefore not involved with, a hypothetical receptor, with the Met sidechain possibly requiring accommodation in a hydrophobic receptor pocket. This situation contrasts with VI, in which both sidechains are on the 'receptor' side of the backbone, with the likelihood of both a hydrophilic binding group on the receptor for the acidic Asp group, and the hydrophobic region mentioned previously.

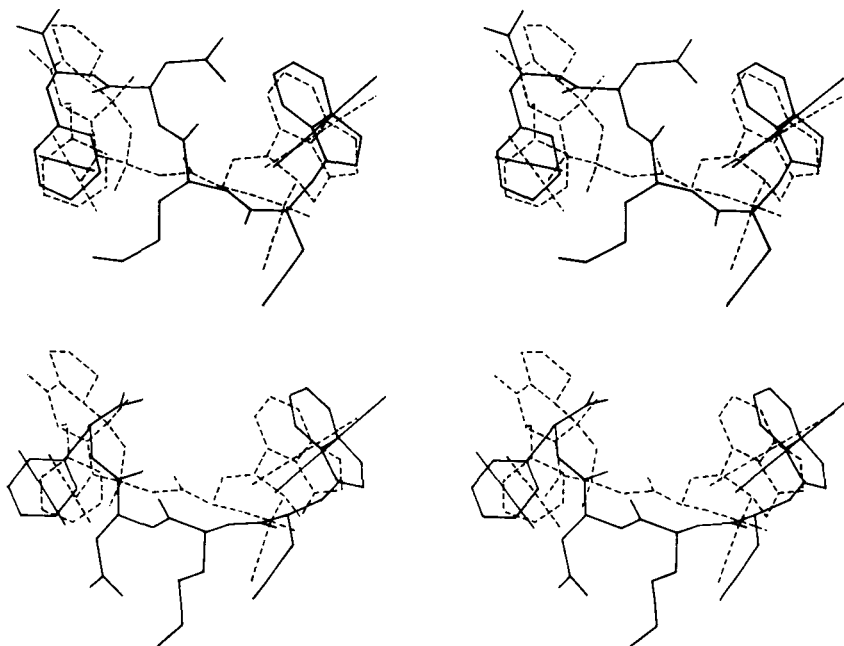


FIG. 2. The two best CCK-4 conformations found (II, top and VI, bottom) shown (solid lines) superimposed on EA (dotted lines) in stereo. Located at the ends of the perpendiculars passing through the centre of the plane of each phenyl and indole ring, and emanating from the positively charged nitrogen atom of both CCK-4 and EA, are the receptor guidepoints used in the fitting procedure (see text).

## CONCLUSION

Beginning with a crystal structure on the one hand, and Dreiding models and a multistage, computer-assisted selection procedure on the other, it was possible to identify two low-energy conformations of CCK-4 capable of mimicking ergotamine. Given this and the pharmacological evidence stated above, it is probable that CCK and the ergopeptides do share common sites of action in the CNS.

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