Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 9-16

MODELS FOR THE A- AND B-RECEPTOR-BOUND CONFORMATIONS OF CCK-8

Gregory V. Nikiforovich# and Victor J. Hruby*

Department of Chemistry, University of Arizona, Tucson, AZ 85721

Received May 19, 1993

Summary: Energy calculations were performed for CCK-8 (Asp^{26}-$Tyr(SO_3)^{27}$-Met^{28}-Gly^{29}-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2, **I**) and [desaminoTyr$(SO_3)^{27}$, $Nle^{28,31}$]CCK-7 (**II**), which are nonselective ligands of CCK receptors, and for the CCK-A selective analog [desaminoTyr$(SO_3)^{27}$, $Nle^{28,31}$, N-Me-Asp^{32}]CCK-7 (**III**) and the CCK-B selective analog [desaminoTyr$(SO_3)^{27}$, Nle^{28}, N-Me-Leu^{31}]CCK-7 (**IV**). The geometrical shapes of the obtained low energy backbone conformers were then compared with each other, searching for similar spatial arrangements of specific atomic centers. The comparisons were performed separately for peptides with high affinity towards CCK receptors of the A type (compounds **I**, **II** and **III**) and for peptides with high affinity towards CCK receptors of the B type (compounds **I**, **II** and **IV**). Possible models for CCK "A"- and "B"-receptor-bound conformations were then developed. The proposed CCK "B-conformation" has a distorted β–III turn at the C-terminal Gly-Trp-Met-Asp fragment, the Phe^{33} residue and the C-terminal amide being directed outward from the turn. The CCK "A-conformation" has two reversals of the peptide chain so that the C^{α}-atoms of the C-terminal pentapeptide appear at the corners of a nearly regular pentagon, and a distinct β–II turn is centered at the N-terminal Tyr-Met-Gly-Trp fragment, the planes of the turn and the pentagon being almost perpendicular. The proposed models are consistent with the results of biological testing for CCK related peptides including cyclic analogs and CCK-A selective tetrapeptides.

It has been proposed that the C-terminal octapeptide of the brain and gut peptide cholecystokinin H-Asp^{26}-$Tyr(SO_3)^{27}$-Met^{28}-Gly^{29}-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2 (CCK_{26-33}, CCK-8) interacts specifically with at least two different receptor types, which have been referred to as the A (peripheral) and B (brain) receptors [1,2]. Receptors of the A type have high affinity for CCK-8, but not for the unsulfated form of CCK-8, or for the C-terminal carboxylated tetrapeptide of CCK (CCK-4), or for gastrin. On the other hand, receptors of the B type have approximately the same high affinity for all of the above mentioned peptides. Generally, the binding potency towards A receptors and corresponding biological activities of analogs are reduced as a result of modifications of

#Present address: Center for Molecular Design, Washington University, St. Louis, MO 63130.

*To whom reprint requests should be addressed.

0006-291X/93 $4.00

the side chains of the Tyr^{27}, Trp^{30}, Asp^{32} and Phe^{33} residues. Receptors of the B type seem to require primarily the C-terminal tetrapeptide sequence to elicit high affinity and a full biological response.

The conformation-bioactivity relationships for CCK receptors of the A and B types also may be different. However, no models of receptor-bound conformations of CCK-8 have been proposed specifically for the A and B receptors. The available data on possible CCK-8 or CCK-7 (CCK_{27-33}) conformations are limited to those derived from fluorescence measurements [3], NMR [4, 5], NMR followed by energy minimization [6], NMR combined with fluorescence measurements and energy calculations [7], and energy calculations alone [8-10]. Recently, several highly selective CCK analogs with N-methyl-amino acid residues were synthesized in our laboratory [11] and elsewhere [12]. These findings offer an opportunity to propose separate models of the receptor-bound conformers of CCK-8 for the A and B type receptors by separate comparison of the sets of low-energy structures of analogs with high affinities toward A or B receptors, respectively.

With this in mind, four CCK-related compounds were chosen in this study for energy calculations and subsequent comparison of geometrical shapes. Two of them, namely CCK-8 itself (compound **I**, IC_{50} values are 0.13 and 0.32 nM for A and B receptors, respectively [11,12]) and desaminoTyr(SO_3)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (compound **II**, IC_{50} values being 0.77 and 0.50 nM [11,12]) represent CCK peptides with high affinities towards both A and B type receptors. Compound **III** (desaminoTyr(SO_3)-Nle-Gly-Trp-Nle-N-Me-Asp-PheNH_2) has high selectivity towards CCK-A receptors (IC_{50} values are 0.42 and 300.0 nM [12]), and compound **IV** (desaminoTyr(SO_3)-Nle-Gly-Trp-N-Me-Nle-Asp-Phe-NH_2) is highly selective towards CCK-B receptors (IC_{50} values are 110.0 and 0.19 nM [12]).

METHODS

The methods for conformational search and energy calculations were basically the same as described previously [13]. The ECEPP potential field [14] was used for conformational energy calculations assuming rigid valence geometry with a planar *trans*-peptide bonds (both *trans* and *cis* peptide bonds were examined for N-Me-amino acid residues; in these cases the ω angle also was allowed to rotate). The valence geometry and atomic charges at the negatively charged SO_3^- group were calculated by the use of the SYBYL program. Aliphatic and aromatic hydrogens were generally included in united atomic centers of CH_n type; only H^α-atoms were described explicitly. The main calculation scheme for all compounds consisted of a build-up procedure of stepwise successive "growing" of the peptide chain toward the N-terminus starting from C-terminal pentapeptides. At the first step of the calculations, all possible combinations of local minima for the peptide backbone for each amino acid residue were considered. Generally, these minima were of *E*, *F*, *C*, *A* and *A** types (according to the notation in

[15]) for L-amino acid residues, and of *E**, *F**, *C**, *A** and *A* type for D-amino acid residues. For Gly residues, minima of *E*, *F*, *C*, *A*, *A**, *C**, *F** and *E** types were considered. The *A*-type minimum was omitted for residues preceding N-Me-amino acids. From low energy structures obtained at the previous step only those differing by more than 60° in at least one value of any backbone dihedral angle were selected for the next step. The backbone structures selected at the previous step by $E - E_{min} < \Delta E$ criteria were considered at subsequent steps. The dihedral angle values of side chain groups and of the terminal groups of the backbone (including those of desaminoTyr(SO_3)) were optimized at every step before energy minimization to achieve their most favorable spatial arrangements according to an algorithm described in [13].

Geometric comparison of a pair of conformers belonging to different molecules included an assessment of the best fit (according to [16]) of the space arrangement of the atomic centers chosen to represent the most important elements of CCK interactions with A or B receptors. Two conformers were regarded as geometrically similar when the corresponding **rms** value was less than 1.0 Å.

RESULTS

Following extensive search procedure, we obtained 23 low energy structures (DE < 1 kcal/mol per residue) of the peptide backbone for compound **I**, 12 for **II**, 109 for **III** and 17 for **IV**. It is noteworthy that all low energy conformers for the N-Me-Nle-containing compound **IV** possess a *trans* conformation of the corresponding peptide bond ($\omega \sim 180° \pm 35°$), while there are 5 low energy conformers for compound **III** with a *cis* conformation of the N-Me-Asp residue.

The models of receptor-bound conformations for A receptors should be looked for among geometrically similar low energy conformers of compounds with high affinity for A receptors (i.e. compounds **I**, **II** and **III**), and the same for compounds **I**, **II** and **IV** in the case of B receptors. On the basis of structure-activity data, seven atomic centers were selected to represent the structural elements required for binding towards A receptors, namely the C^{α}-atom of Tyr^{27}, and the C^{α}- and C^{β}-atoms of the Trp^{30}, Asp^{31} and Phe^{33} residues. The spatial arrangements of these centers were then compared for each pair of low energy structures obtained for compounds **I**, **II**, and **III**. The lowest energy conformers among those bearing geometrically similarity for all three compounds ("A-conformers") are listed in the first part of Table 1. The resulting model of "A-conformer" for CCK-8 is composed of two peptide chain reversals (see Fig. 1). First, there is a distinct β–II turn at the Tyr-Met-Gly-Trp fragment, stabilized by a hydrogen bond of the Trp(NH)...(OC)Tyr type (Fig. 1b). Secondly, the C^{α}-atoms of C-terminal pentapeptide Gly-Trp-Met-Asp-Phe are located approximately in the same plane near the corners of an almost regular pentagon (Fig. 1c). The plane of this pentagon is oriented almost perpendicular to the plane of the β-turn described above. Thus, the Gly-Trp fragment participates in both chain reversals.

Table 1. Models for A- and B-conformers of peptide backbones for compounds I-IV

Residue:	Asp	Tyr		Met/Nle		Gly		Trp		Met/Nle/NMeLeu			Asp/NMeAsp			Phe
Angle: Comp.	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ω	ϕ	ψ	ω	ϕ
							A-conformers									
I	140	-159	-57	-77		112	107	3	-134	-58	-98	-47	-	-104	149	-	-141
II	-	-	-103	-74		132	104	-3	-140	-57	-97	-51	-	-97	153	-	-145
III	-	-	116	-48		121	119	-46	-99	-44	-136	122	-	60	149	-145	-152
							B-conformers									
I	148	-144	47	-51		125	106	-38	-88	-40	-68	-28	-	-80	78	-	-80
II	-	-	127	-63		118	91	9	-130	-67	-115	-8	-	-122	135	-	-140
IV	-	-	82	-128		44	121	-13	-149	140	54	32	-170	-94	-33	-	52

In order to find "B-conformers" we searched for geometrical similarity between low energy backbone conformers of compounds **I, II** and **IV**. Five atomic centers were selected for comparison in this case, namely the C^{α}-atoms of Trp^{30}, Met/Nle/N-Me-Leu^{31}, Asp^{32} and Phe^{33}, and the C^{β}-atom of Met/Nle/N-Me-Leu^{31}, representing the peptide backbone of the C-terminal tetrapeptide. The lowest energy, geometrically similar, backbone conformers obtained for "B-conformers" are listed in the second part

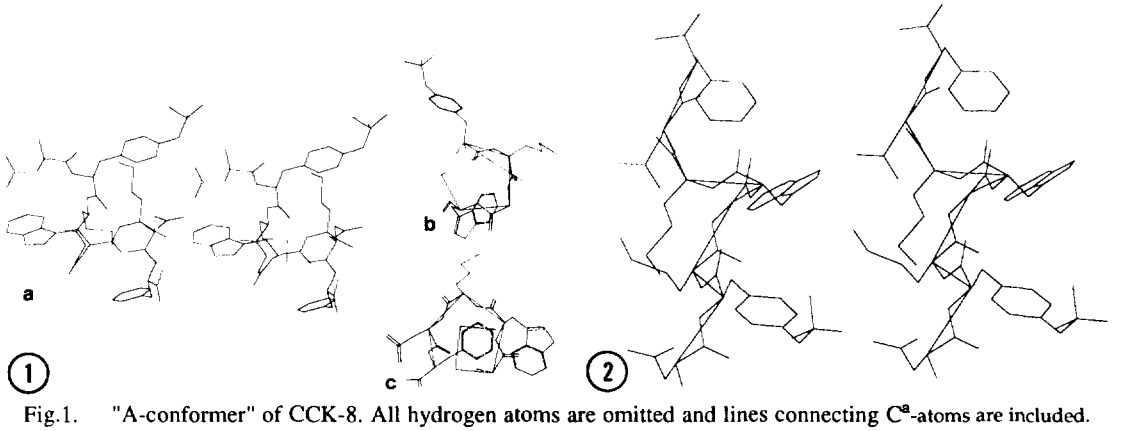

Fig.1. "A-conformer" of CCK-8. All hydrogen atoms are omitted and lines connecting C^{α}-atoms are included. a) The general view of conformation; b) the β-II turn in the Tyr(SO_3)-Met-Gly-Trp fragment, and, c) the "pentagon" structure in the Gly-Trp-Met-Asp-Phe fragment. Only fragments in question are depicted for b) and c) parts.

Fig.2. "B-conformer" of CCK-8. All hydrogen atoms are omitted and lines connecting C^{α}-atoms are included.

of Table 1. Only conformations of the C-terminal tetrapeptide portions of each compound are significant in this case, so "B-conformers" of CCK-8 are, in fact, the conformers of the C-terminal tetrapeptide only. In Fig. 2 we observe a characteristic β-III turn in "B-conformer" including the Gly-Trp-Met-Asp sequence and stabilized by a distorted hydrogen bond of the Asp^{32}(NH)...(OC)Gly^{29} type. The side chain of the Asp^{32} residue is directed towards the Gly^{29} residue, facilitating a possible hydrogen bond of the Gly(NH)...(βOOC)Asp type. The Phe^{33} residue and the C-terminal amide are directed outward from the β-turn.

DISCUSSION

Previously, a few authors have performed energy calculations searching for low energy structures of CCK-8 [6-10]. The authors of [10] based their calculations on the assumption that the backbone structure of the C-terminal tetrapeptide is almost fully extended, as was found in the X-ray structure of tetragastrin [17]. None of the low energy structures of CCK-8 obtained in our calculations possess such a feature. The paper [6] presented two calculated CCK-8 structures which were thought to correspond to the lowest energy conformer and a conformer most compatible with NMR data, respectively. When our energy calculations (see *Methods* section) were applied to these structures, their energies were found to be 18.6 and 16.1 kcal/mol, respectively, compared to the lowest energy conformer of CCK-8 obtained in the present study.

The investigations in [7,8] thoroughly explored the conformational space for CCK-8 backbone. These calculations were based on Monte-Carlo simulations with fairly large numbers (a total of 10^6) of generated conformations. The calculations of Fournie-Zaluski et al. [7] resulted in 5 low energy backbone structures. All possess relative energies of more than 8 kcal/mol when they were re-calculated by the methods used here. The same re-calculation procedures were performed for the 6 low energy backbone structures proposed by Kreissler et al. [8]. In two cases, the relative energies obtained were less than 8 kcal/mol, namely, 6.0 kcal/mol for structure 4 and 3.7 kcal/mol for structure 6 (structure numbering according to Table 3 in [8]). A comparison of the geometrical shapes between the structures proposed in [8] and those found in this study (the space arrangements of all C^{α}-atoms were compared) revealed that two structures from our list of low energy conformers are similar to structure 4 from [8], but none of our structures are similar to structure 6 in [8]. Nevertheless, the results of our energy calculations should be considered to be more comprehensive, since they found a much larger set of low energy geometrical forms for the CCK-8 peptide backbone.

The proposed "A-conformer" of CCK-8 possesses an N-terminal β-turn in the Tyr-Met-Gly-Trp fragment instead of the Asp-Tyr-Met-Gly sequence as was suggested in [5,7]. This difference explains the lack of affinity towards A receptors for conformationally constrained cyclic analogs such as Boc-X-Tyr(SO_3)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2 (X = D-Asp, γ-D-Glu) which were designed for the purpose of stabilizing the N-terminal β-turn at the Asp-Tyr-Nle-Gly sequence [18]. At the same time, the C-terminal tetrapeptide remains untouched in these analogs, preserving the ability to adopt the "B-conformer", which leads to B-selectivity of these cyclic compounds [18]. Cyclic analogs of another type, namely

X-Lys-Gly-Trp-Lys-Asp-Phe-NH_2 (X = Ac-Tyr(SO_3), Ac-Tyr, Ac, H)
└CO-$(CH_2)_n$-CO┘

also were shown to be B-selective [19]. In this case, the C^{α}-atoms of the Lys residues are involved in a 24-membered ring. A chain of such length perhaps would be sufficient to close the ring without significant distortions of the "B-conformer" of the C-terminal tetrapeptide (see Fig. 2). Thus far there are no cyclic analogs of CCK-8 displaying selectivity towards CCK-A receptors, and all of the cyclic analogs in [18,19] are incompatible with the "A-conformers" proposed in the present study.

The CCK related analogs with substitutions of the conformationally flexible Gly residue for either an L- or D-amino acid residue also can be regarded as conformationally restricted. According to Table 1, the Gly^{29} residue adopts conformations corresponding to the right half of the Ramachandran plot both for "A"- and "B-conformers" of compounds **I** - **IV**. The local energy minima in this region are more preferable when Gly is substituted by D-Ala, than by L-Ala. Indeed, the CCK biological activity related to binding towards A receptors remains of the same order for [D-Ala^{29}]CCK-8 [5,20] and Ac [D-Ala^{29}]CCK-7 [21], but drops dramatically in the case of Boc-[Ala^{29}]CCK-7 [5,20]. These findings can also be explained in terms of preserving/changing the "A-conformers" for corresponding CCK analogs. Also, quite recently, a Pro^{28}-containing analog of compound **II** was found to be both A- and B-potent [22], which is in good agreement with the proposed ϕ value (~ -60°) for Nle^{28} residue of compound **II** (see Table 1).

The above data on the biological activities of conformationally restricted CCK analogs constitute substantial, though indirect, evidence for the models of the "A-" and "B-conformers" of CCK-8 proposed here. Thus, it became interesting to compare the proposed "A-conformers" with the possible structures for the recently reported A-selective tetrapeptide, Boc-Trp-Lys(ε-NHCONH-*o*-Me-Phe)-Asp-Phe-NH_2 (compound **V**,

IC_{50} values are 3.8 and 1500.0 nM for A and B receptors, respectively) [23]. The energy calculations for compound **V** were performed in the same way as for compounds **I - IV**. The Boc group was substituted by an acetyl group in these calculations. All combinations of rotamers g^+, t and g^- for dihedral angles χ_1-χ_4 of the Lys residue were considered for every backbone conformer. The resulting low energy structures of compound **V** were compared then with the "A-conformers" of CCK-8 taking into account the C^α- and C^β-atoms of the Trp^{30}, Asp^{32} and Phe^{33} residues. It was found that several low energy conformations of **V** indeed are similar to the "A-conformers" of CCK-8 with the **rms** values less than 1.0 Å. Therefore, the aromatic rings of the Tyr(SO_3) residue and of the Lys(εNHCOHN-*o*-Me-Phe) residues can occupy nearly the same spatial positions.

In summary, the "A-" and "B-conformers" of CCK-8 proposed in this study appear to elucidate the conformational factors that lead to receptor selectivity (or the loss of it) for several A- or B-selective CCK agonist analogs, including a "non-traditional" tetrapeptide analog **V**. The last case clearly demonstrates that the topographical features of CCK agonists (i.e. the spatial arrangement of functionally important groups), rather than the conformational ones (e.g. the torsional angles) are crucial for receptor binding (see also [13]). As for CCK antagonists and inhibitors, the topographical features crucial for their binding to CCK receptors might be different (see, e.g. [24,25]).

ACKNOWLEDGMENTS

This work was supported in part by grants from the U. S. Public Health Service DK 36289, and DK 17420, and by a grant from the National Science Foundation.

REFERENCES

1. Innis, R.B., and Snyder, S.N. (1980) *Proc. Natl. Ac. Sci. USA*; **77**, 6917-6921
2. Sakamoto, C., William, J.A., and Goldfine, I.D. (1984) *Biochem. Biophys. Res. Commun.*; **124**, 497-502
3. Schiller, P.W., Natarajan, S., and Bodanszky, M. (1978) *Int. J. Peptide Protein Res.*; **12**, 139-142
4. Durieux, C., Belleney, J., Lallemand, J.-Y., Roques, B.P., and Fournie-Zaluski, M.-C. (1983) *Biochem. Biophys. Res. Commun.*; **114**, 705-712
5. Gacel, G., Durieux, C., Fellion, E., Fournie-Zaluski, M.C., Begue, B., Menant, I., Rossignol, P., and Roques, B.P. (1984) in *Peptides 1984. Proc. of the 18th Eur. Peptide Symp.* (Ragnarsson, U., ed.), pp. 383-385, Almquist and Wiksell Int., Stockholm
6. Loomis, R.E., Lee, P.-C., and Tseng, C.-C. (1987) *Biochim. Biophys. Acta*; **911**, 168-179
7. Fournie-Zaluski, M.C., Belleney, J., Lux, B., Durieux, C., Gerard, D., Gacel, G., Maigret, B., and Roques, B.P. (1986) *Biochemistry*; **25**, 3778-3787
8. Kreissler, M., Pesquer, M., Maigret, B., Fournie-Zaluski, M.C., and Roques, B.P. (1989) *J. Computer-Aided Mol. Design*; **3**, 85-94
9. Pincus, M.R., Carty, R.P., Chen, J., Lubowsky, J., Avitable, M., Shah, D., Scheraga, H.A., and Murphy, R.B. (1987) *Proc. Natl. Acad. Sci. USA*; **84**, 4821-4825
10. Coats, E.A., and Knittel, J.J. (1990) *Quant. Struct. Act. Relat.*; **9**, 94-101

11. Hruby, V.J., Fang, S., Knapp, R., Kazmierski, W., Lui, G.K., and Yamamura, H.I. (1990) *Int. J. Peptide Protein Res.*; **35**, 566-573
12. Lin, C.W., Holladay, M.W., Witte, D.G., Miller, T.R., Wolfram, C.A.W., Bianchi, B.R., Bennett, M.J., and Nadzan, A.M. (1990) *Am. J. Physiol*; **258**, G648-G651
13. Nikiforovich, G.V., Hruby, V.J., Prakash, O., and Gehrig, C.A. (1991) *Biopolymers*; **31**, 941-955
14. Dunfield, L.G., Burgess, A.W., and Scheraga, H.A. (1978) *J. Phys. Chem.*; **82**, 2609-2616; Nemethy, G., Pottle, M.S., and Scheraga, H.A. (1983) *J. Phys Chem*; **87**, 1883-1887
15. Zimmerman, S.S., and Scheraga, H.A. (1977) *Biopolymers*; **16**, 811-843
16. Nyburg, S.C. (1974) *Acta Crystallogr.*: **B30 (part I)**, 251-253
17. Cruse, W.B.T., Egert, E., Viswamitra, M.A., and Kennard, O. (1982) *Acta Crystallogr.*; **B38**, 1758-1764
18. Charpentier, B., Pelaprat, D., Durieux, C., Dor, A., Reibaud, M., Blanchard, J.-C. and Roques, B.P. (1988) *Proc. Natl. Acad. Sci. USA*; **85**, 1968-1972
19. Rodriguez, M., Lignon, M.-F., Galas, M.-C., Amblard, M., and Martinez, J. (1990) *Mol. Pharmacology*; 38, 333-341
20. Fournie-Zaluski, M.C., Belleney, J., Durieux, C., Gacel, G., Roques, B.P., Begue,D., Menant, I., Lux, B., and Gerard, D. (1985) *Ann. N.Y. Acad. Sci.*; **448**, 598-600
21. Penke, B., Hajnal, F., Lonovics, J., Holzinger, G., Kadar, T., and Telegdy, G. (1984) *J. Med. Chem.*; **27**, 845-849
22. Holladay, M.W., Bennett, M.J., Tufano, M.D., Lin, C.W., Asin, K.E., Witte, D.G., Miller,T.R., Bianchi, B.R., Nikkel, A.L., Bednarz, L., and Nadzan, A.M. (1992) *J. Med. Chem.*; **35**, 2920-2928
23. Shiosaki, K., Lin, C.W., Kopecka, H., Craig, R., Bianchi, B., Miller, T., Witte, D., Stashko, M. and Nadzan, A.M. (1992) *J. Med. Chem.*; **35**, 2007-2014
24. Gonzalez-Muniz, R., Bergeron, F., Marseigne, I., Durieux, C., and Roques, B.P. (1990) *J. Med. Chem.*; **33**, 3199-3204
25. Hruby, V.J. (1987) *Trends Pharm. Sci*; **8**, 336-339