Additions and Corrections

1992, Volume 35

Jun Segawa, Masahiko Kitano, Kenji Kazuno, Masato Matsuoka, Ichiro Shirahase, Masakuni Ozaki, Masato Matsuda, Yoshifumi Tomii, and Masahiro Kise*: Studies on Pyridonecarboxylic Acids. 1. Synthesis and Antibacterial Evaluation of 7-Substituted-6-halo-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic Acids.

Page 4728. In Scheme I, the reaction condition used in converting **28a-j** to **29a-y** was h, not g. Also, the substituents for **28a-j** should be R^3 = Et, R^7 = cyclic amino, and for **29a-y**, R^3 = H, R^7 = cyclic amino.

Page 4730. In the second sentence of the third full paragraph of the second column, the order of in vitro activity against Gram-positive bacteria should read **29h > 29a > 29e, 29f > 29b, 29c, 29d > 29g.**

1993, Volume 36

Kristin R. Snyder, Thomas F. Murray, Gary E. DeLander, and Jane V. Aldrich*: Synthesis and Opioid Activity of Dynorphin A-(1-13)NH_2 Analogues Containing *cis*- and *trans*-4-Aminocyclohexanecarboxylic Acid.

Page 1101. The first eight lines of text were repeated from the previous page, and the last nine lines of text are missing. For clarity, the sections of text involved are reproduced below.

Results and Discussion

Design Rationale and Synthesis. Molecular modeling with the AMBER[15,16] program was used to examine possible conformational constraints for incorporation into positions 2 and 3 in Dyn A-(1-13)NH_2. These studies suggested that *trans*-4-aminocyclohexanecarboxylic acid (*trans*-ACCA) might replace Gly^2-Gly^3 in an extended conformation. The calculated nitrogen-carbonyl carbon distance was 5.70 Å for *trans*-ACCA in the diequitorial conformation vs 6.12 Å between the nitrogen of Gly^2 and the carbonyl carbon of Gly^3 when the peptide was in an extended conformation. The ACCA dipeptide replacement is equivalent to constraining ψ_2 and ϕ_3 while still allowing free rotation around ϕ_2 and ψ_3.

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-R

Dyn A-(1-13)NH_2

[*trans*-ACCA^{2,3}]Dyn A-(1-13)NH_2

R = -Pro-Lys-Leu-LysNH_2

ACCA was synthesized by hydrogenation of *p*-aminobenzoic acid with PtO_2 as a catalyst[17] (Scheme I), which yielded a mixture of cis and trans isomers (approx ratio of 2.5/1 cis/trans). Separation by fractional recrystallization[18] from EtOH yielded the cis isomer, which was pure by ^1H NMR. Subsequent recrystallization from EtOH/ether yielded the trans isomer, but ^1H NMR indicated that the trans isomer contained from 10-15% up to 35% of the cis isomer, depending on the batch. Following fractional recrystallization, each isomer was converted separately to its Fmoc (9-fluorenylmethoxycarbonyl) derivative. Fmoc protection of these branched amino acids proved to be difficult, and literature procedures[19] had to be modified[20] to obtain the desired product (see Experimental Section). HPLC verified that Fmoc-*cis*-ACCA contained only the cis isomer. In the case of Fmoc-*trans*-ACCA, the contaminating cis isomer (t_R = 29.3 min) was not well resolved from the desired Fmoc-*trans*-ACCA (t_R = 29.7 min) by HPLC, so this mixture was used in the synthesis of the *trans*-ACCA analogue of Dyn A-(1-13)-NH_2.

Both the cis and trans isomers were incorporated separately into Dyn A-(1-13)NH_2 (Scheme I). The peptides were prepared as amides because of the enhanced metabolic stability of Dyn A-(1-13) amide vs its corresponding acid.[21] The peptides were synthesized on a PAL resin[22] using Fmoc-protected amino acids by procedures described previously.[23] The peptides were deprotected and cleaved from the PAL resin using trifluoroacetic acid (TFA) and purified by preparative reverse-phase HPLC. The purification of [*cis*-ACCA^{2,3}]Dyn A-(1-13)NH_2 was straightforward, while the purification of the trans analogue proved difficult due to the necessity of separating

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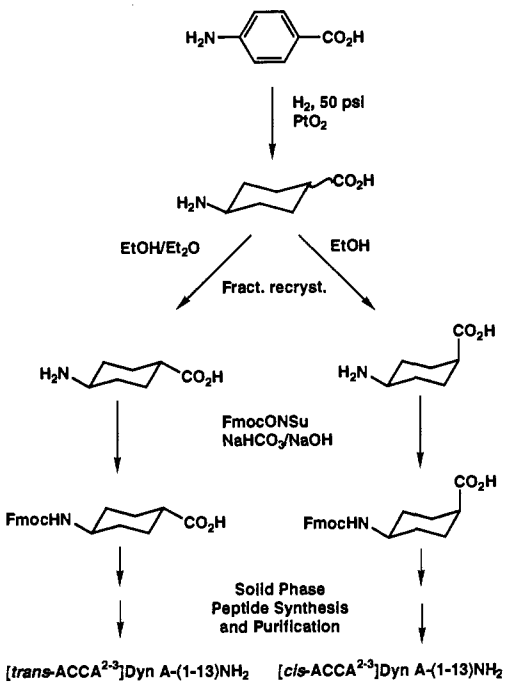
Scheme I. [*trans*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 [*cis*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2



it from the contaminating cis analogue. This resulted in a very low yield of pure [*trans*-ACCA$^{2-3}$]Dyn A-(1–13)-NH_2.

Opioid Receptor Binding Affinities and Opioid Activity. The peptides were evaluated for opioid receptor affinity at κ receptors by measuring the inhibition of binding of [^{3}H]bremazocine to guinea pig cerebellar membranes, and for μ and δ receptor affinities in rat forebrain membranes using [^{3}H]DAMGO ([D-Ala^2,-MePhe^4,glyol]enkephalin) and [^{3}H]DPDPE ([D-Pen^2,D-Pen^5]enkephalin), respectively (Table I).

Both [*cis*-ACCA$^{2-3}$]- and [*trans*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 bound to κ opioid receptors with modest affinity (K_i = 9–13 nM), with the *cis*-ACCA analogue exhibiting slightly greater affinity than the trans isomer. The affinity of these analogues for κ receptors is $^1/_{60}$ to $^1/_{90}$ that of the parent peptide Dyn A-(1–13)NH_2. Introduction of either isomer of ACCA into Dyn A-(1-13)NH_2 causes an even larger decrease in affinity for μ receptors, resulting in K_i's greater than 100 nM. Therefore, both of these analogues are κ selective, with [*trans*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 showing slightly better κ selectivity (κ/μ ratio = 1/21) than [*cis*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 (κ/μ ratio = 1/13). Both of these peptides had very little affinity for δ receptors (K_i's > 1000 nM).

Both the *cis*- and *trans*-ACCA Dyn A analogues show binding affinity similar to the Dyn A-(1–13)NH_2 analogues containing an L-amino acid at position 2 reported by Story et al.,[24] all of which had K_i's in the 2–20 nM range. The ACCA-substituted peptides, however, have better κ vs μ selectivity than any of these 2-substituted dynorphin analogues. [Aib^2]Dyn A-(1–13),[25] which incorporates an α,α-disubstituted amino acid into position 2 similar to the disubstitution α to the amine of ACCA, surprisingly has lower affinity for κ receptors (K_{i_κ} = 50 nM). [Aib^2]Dyn A-(1–13) has 10–30-fold higher affinity for μ receptors (K_{i_μ} = 10 nM) than [*cis*- and *trans*-ACCA$^{2-3}$]Dyn A-(1–13)-NH_2; thus, the Aib^2-substituted peptide is μ selective while the ACCA-substituted peptides are κ selective.

[*cis*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 was evaluated for opioid activity in the guinea pig ileum (GPI) assay. Its potency (IC_{50} = 4.09 μM, 95% confidence = 2.88–5.80 μM) was much lower than the parent Dyn A-(1–13)NH_2 (IC_{50} = 0.24 nM, 95% confidence = 0.21–0.29 nM), but this analogue exhibited a full dose–response curve and naloxone antagonized its effects (data not shown). The low GPI activity of [*cis*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 parallels the results obtained for the analogues containing L-amino acids in position 2.[24] There was insufficient trans compound to allow for its testing in the GPI.

Conclusions

[*cis*- and *trans*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 are the first reported Dyn A analogues conformationally constrained in the "message" sequence that are selective for κ opioid receptors. The *cis*- and *trans*-ACCA$^{2-3}$-substituted peptides showed surprisingly similar κ, μ, and δ affinities when compared to each other and to Dyn A analogues containing an L-amino acid in position 2.[24] These results suggest that the Gly^2-Gly^3 peptide bond is not critical for κ opioid receptor affinity or selectivity. The similar binding affinities of the *cis*- and *trans*-ACCA Dyn A analogues, however, make it difficult to make conclusions concerning the bioactive conformation of the "message" sequence of Dyn A. The differences observed in receptor affinities and selectivity between the κ-selective [*cis*-ACCA$^{2-3}$]- and [*trans*-ACCA$^{2-3}$]Dyn A-(1–13)NH_2 and the μ-selective Dyn A analogue [Aib^2]Dyn A-(1–13)[25] could be due to differences in conformation, the larger size of ACCA vs Aib, or the substitution at the position corresponding to the C_α of Gly^3 in the ACCA-substituted peptides.

The good discrimination of κ vs μ receptors exhibited by the *cis*- and *trans*-ACCA derivatives of Dyn A-(1–13)-NH_2 is encouraging and suggests that further modification at positions 2 and/or 3 might lead to Dyn A analogues with higher κ receptor affinity and opioid potency.

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