Highly κ Receptor-Selective Dynorphin A Analogues with Modifications in Position 3 of Dynorphin A(1–11)-NH_2

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The heptadecapeptide dynorphin A (Dyn A(1–17); H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH) was first isolated from porcine pituitary and recognized as a potent opioid agonist.[2–4] It is now well established that there are at least three types of opioid receptors, namely, μ, δ, and κ.[5–7] However, the physiological and pharmacological roles of these receptors still need clarification, thus requiring the design and synthesis of highly potent and selective ligands. Research in this area has expanded rapidly in the past decade, with considerable effort devoted to the development of δ and μ opioid receptor-selective peptide ligands primarily based on enkephalin.[8–11] The potential of targeting the κ opioid receptor as an effector for analgesia has yet to be explored in detail.[12] Previous structure–activity studies have shown that the truncated derivative, dynorphin A(1–11)-NH_2, retains the high binding potency of dynorphin A at the κ receptor. Thus we and others have primarily used Dyn A(1–11)-NH_2 as a template to examine the structure–activity relationships of dynorphin.[13,14] Since Tyr[1] and Phe[4] are reported to be important for opioid agonist activity, the effects of the glycine residues in positions 2 and 3 of Dyn A on the relative orientations of the two aromatic rings may be biologically important.[13] To assess these possible effects, we have substituted Gly[3] by D- and L-alanine residues to form the linear peptides [D-Ala[3]]Dyn A(1–11)-NH_2 and [Ala[3]]Dyn A(1–11)-NH_2. We report here that both analogues are very potent for κ receptors and very selective for κ vs μ and κ vs δ receptors. These results suggest that substitution of lipophilic residues and/or certain D-amino acids at position 3 of Dyn A(1–11)-NH_2 may lead to novel Dyn A analogues which exhibit enhanced selectivities for κ receptors, while retaining strong affinities.

Peptide Synthesis and Purification. Dynorphin A analogues were synthesized by solid phase methods that were reported previously for the synthesis of other Dyn A analogues.[14,15] Side chain-protected N^{α}-Boc amino acids were purchased from Bachem (Torrance, CA), whereas the other amino acids were synthesized by standard methods in our laboratory. The synthesized analogues were purified by RP-HPLC (linear gradient of 10–90% acetonitrile in 0.1% TFA in water over 40 min) and characterized by FAB-MS and amino acid analysis. The purity of the synthetic peptides was assessed by TLC (single spot in four different solvent systems, ninhydrin detection) and HPLC (one single peak, UV detection at 280 and 225 nm, using two different linear gradients).

Opioid Receptor Binding Affinities and Selectivities in the Guinea Pig Brain (GPB). The peptides were evaluated for their receptor binding affinities at κ, δ, and μ receptors by measuring the inhibition of binding of [^{3}H]U-69,593 (*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzo[*b*]furan-4-acetamide) (κ), [^{3}H]cyclo[D-Pen[2],*p*-Cl-Phe[4],D-Pen[5]]enkephalin (δ), and [^{3}H]DAMGO ([D-Ala[2],MePhe[4],Glyol[5]]enkephalin) (μ) to opioid receptors in guinea pig brain (GPB) homogenates (Table 1).[14,15] As shown in Table 1, substitution of Gly[3] with D- and L-alanine lead to analogues **2** and **3** that displayed high affinities at the central κ opioid receptor (IC_{50} = 0.76 and 1.1 nM, respectively) similar to that of Dyn A(1–11)-NH_2 (**1**) (IC_{50} = 0.58 nM) and, rather surprisingly, greatly enhanced selectivities for κ vs μ and κ vs δ receptors (IC_{50} ratios of 350 and 1300 for **2** and 190 and 660 for **3**, respectively, compared to 17 and 44 for **1**), due to poor affinities for μ and δ receptors (Table 1). The binding affinity obtained for analogue **3** differs somewhat from one previously published for [Ala[3]]Dyn A(1–13).[16] No satisfactory explanation can be given for this discrepancy except that this latter study used a 1–13 analogue, a different radioligand, and a much shorter incubation time in the binding assay (30 min vs 180 min).

Analogue **2** is one of the most κ receptor-selective dynorphin-like peptides reported and can be compared to [N-benzyl-Tyr[1],D-Pro[10]]Dyn A(1–13)-NH_2, a peptide that exhibits so far the highest reported selectivity for the central κ vs μ and δ receptors ($\kappa/\mu/\delta$ K_i ratio = 1/1070/6080).[17] The fact that a higher κ selectivity can be observed with both analogues **2** and **3**, by incorporating the two alanine enantiomers, suggests that an important factor could be related more to the increase in lipophilicity than to a specific orientation of the methyl group of alanine. Another possibility is that replacing an α-helix-breaking residue like glycine could increase the α-helical content of the message segment of dynorphin A, which has been postulated to be important for κ site selection.[18]

Biological Activities in the Guinea Pig Ileum (GPI). The κ (and μ) opioid activities of these peptides were measured by their ability to inhibit the electrically evoked contraction of the guinea pig ileum (GPI) (Table 2)[14,15] and the effect of the μ opioid receptor antagonist CTAP on the IC_{50} value.[19] The results obtained in GPI bioassay show that, as no shift in activity can be observed upon addition of the μ antagonist CTAP, all analogues tested interact only or specifically with the peripheral κ receptors. Though they are still potent at these opioid receptors, [D-Ala[3]]Dyn A(1–11)-NH_2 and [L-Ala[3]]Dyn A(1–11)-NH_2 display somewhat lower potencies than the standard **1** (IC_{50} = 8.1 and 1.7 nM, respectively, for **2** and **3**, vs 1.1 nM for **1**).

Conclusion. It has been found that substitution of Gly[3] with D- and L-alanine in Dyn A(1–11)-NH_2 (analogues **2** and **3**) resulted in a dramatic increase in selectivity for κ receptors, when compared to Dyn A(1–11)-NH_2. Though it still is not possible to say whether these results are due to (1) an increase in lipophilicity of the peptide, (2) a possible enhancement of the α-hel-

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Table 1. Opioid Receptor Binding Affinities and Selectivities of Dyn A(1−11)-NH_2 Analogues in Guinea Pig Brain (GPB) Homogenate

analogues of Dyn A(1−11)-NH_2	IC_{50} (nM)[a]			selectivity	
	κ	μ	δ	μ/κ	δ/κ
Dyn A(1−11)-NH_2 (1)	0.58 ± 0.03	9.90 ± 2.02	25.5 ± 3.4	17	44
[D-Ala^3]Dyn A(1−11)-NH_2 (**2**)	0.76 ± 0.28	260 ± 57	1000 ± 422	350	1300
[Ala^3]Dyn A(1−11)-NH_2 (**3**)	1.10 ± 0.40	210 ± 40	730 ± 50	190	660

[a] The radioligands used were [^{3}H]U-69,593 (κ receptor), [^{3}H]DAMGO (μ receptor), and [^{3}H]cyclo[D-Pen^2,*p*-Cl-Phe^4,D-Pen^5]enkephalin (δ receptor). Results are given ± SEM.

Table 2. Bioassays with the Smooth Muscle Tissue of the Guinea Pig Ileum (GPI)

analogues of Dyn A(1−11)-NH_2	IC_{50} (nM)	
	GPI	shift[a]
Dyn A(1−11)-NH_2 (1)	1.1 ± 0.3	ns
[D-Ala^3]Dyn A(1−11)-NH_2 (**2**)	8.1 ± 2.3	ns
[Ala^3]Dyn A(1−11)-NH_2 (**3**)	1.7 ± 0.2	ns

[a] ns: no significant shift observed with 1000 nM CTAP used as a μ antagonist. Results are given ± SEM.

ical content of the message segment of this peptide, (3) a more favorable spatial arrangement of the relative positions of the aromatic residues, or (4) a combination of these different effects, they are highly interesting, as they provide us with new lead compounds for further enhancement of potency and selectivity. Several other peptides, incorporating different modifications at position 3, are currently being synthesized in our laboratory, will be examined for their binding affinities and selectivities, and also will be studied by circular dichroism and nuclear magnetic resonance techniques, in order to determine if conformational changes take place in these analogues and if those changes can account for the increased selectivity of these analogues for κ receptors.

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977−983). All optically active amino acids are of the L variety unless otherwise stated. Other abbreviations are Dyn A, dynorphin A; GPB, guinea pig brain; GPI, guinea pig ileum; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; CTAP, cyclo-[D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2].

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