N-Terminal Alkylated Derivatives of [D-Pro[10]]dynorphin A-(1-11) Are Highly Selective for κ-Opioid Receptors

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Dynorphin A (Dyn A), one of the endogenous opioid peptides, preferentially interacts with κ-opioid receptors[1] and is therefore thought to be an endogenous ligand for this opioid receptor type. Since it also exhibits good affinity for μ- and δ-opioid binding sites,[2] various structural modifications have been made to Dyn A in attempts to identify analogues with enhanced selectivity for κ-receptors. Such analogues could then be used as tools to better understand the physiological effects of Dyn A mediated by κ-receptors. It has been reported that the introduction of *N,N*-diallyltyrosine at position 1 of [D-Pro[10]]Dyn A-(1-11) endows the peptide with antagonist activity. *N,N*-Diallyl-[D-Pro[10]]Dyn A-(1-11), however, exhibits low selectivity for κ-receptors and is only a weak antagonist.[3] As part of our efforts to develop selective and potent antagonists for κ-receptors, we synthesized N-monoalkylated [D-Pro[10]]Dyn A-(1-11) analogues in order to examine whether the second alkyl group was necessary for antagonist activity. Surprisingly, the introduction of an N-monoalkylated tyrosine at position 1 provided marked selectivity for κ-opioid binding sites. The agonist potency of these peptides in the guinea pig ileum (GPI) assay varied depending on the N-terminal substituents.

R-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-D-Pro-LysOH

R = allyl, cyclopropylmethyl (CPM), or benzyl

The peptides were prepared by solid-phase peptide synthesis. Resin-bound [D-Pro[10]]Dyn A-(2-11) was assembled on a PAC resin (Millipore, Marlborough, MA) using Fmoc-amino acids.[4] N-Monosubstituted tyrosine derivatives were synthesized from tyrosine *tert*-butyl ester by treatment with an equimolar amount of the corresponding alkyl halides (allyl chloride, cyclopropylmethyl bromide, or benzyl chloride) and diisopropylethylamine.[5] The *N,N*-diallyltyrosine derivative was prepared in an analogous fashion using an excess of allyl bromide. Following removal of the *tert*-butyl groups with TFA/anisole (9:1), the alkylated tyrosine derivatives were coupled to the assembled peptide chain using BOP, 1-hydroxybenzotriazole, and *N*-methylmorpholine.[6] The peptides were cleaved from the support using TFA/H_2O (95:5) and purified by preparative reverse-phase HPLC.

The peptides were examined for their affinity for opioid receptors in radioligand binding assays (Table I) and for their opioid activity in the GPI assay (Table II). As shown in Table I, the N-monoalkylated analogues retained κ-receptor affinity similar to that of the parent peptide. While [D-Pro[10]]Dyn A-(1-11)[8] exhibited only modest κ-receptor selectivity in our radioligand binding assays, N-monoalkylation of this peptide markedly decreased affinities for μ- and δ-opioid binding sites, resulting in exceptionally κ-selective peptides. Introduction of a second allyl group on the amine terminus of *N*-allyl-[D-Pro[10]]Dyn A-(1-11) to give the *N,N*-diallyl analogue,[3] however, decreased κ-receptor affinity 73-fold while μ-receptor affinity decreased only by a factor of 3, resulting in a marked decrease in κ-receptor selectivity.

In the GPI assay (Table II), the *N*-allyl and *N*-CPM analogues were moderately potent agonists, while the *N*-benzyl and *N,N*-diallyl analogues were very weak agonists. Low pA_2 values for naloxone against the *N*-allyl and *N*-CPM derivatives are in agreement with the κ-receptor selectivity of these analogues observed in the radioligand binding assays and consistent with the pA_2 value observed for U-50,488. The weak agonist activity of the *N*-benzyl analogue was unexpected, given this peptide's high affinity for κ-receptors in the guinea pig cerebellum and the potency of the other two N-monoalkylated derivatives in the GPI. The *N*-benzyl analogue was examined for antagonist activity in the GPI, but statistically significant antagonism could not be detected when it was tested at doses up to 1 μM against Dyn A-(1-13) amide. We are investigating the *N*-benzyl analogue further to attempt to explain its anomalous behavior.[10] The weak partial agonist activity observed for the *N,N*-diallyl analogue is in contrast to the absence of agonism reported for this compound by Gairin et al.[3,11] but consistent with the weak agonist activity reported for the related *N,N*-diallyl-Dyn A-(1-13).[12]

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(4) The amino acids were coupled to the growing peptide chain using diisopropylcarbodiimide and *N*-hydroxybenzotriazole in *N,N*-dimethylacetamide (DMA). Side-chain-protecting groups used were (2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl (Pmc) for arginine and Boc for lysine. The Fmoc protecting group was removed with a 30% piperidine, 35% toluene, and 35% DMA solution.

(5) The reaction was terminated before complete consumption of starting material (after 37-70 h at room temperature) to avoid formation of dialkylated products. The desired products were obtained in 63-83% yield, together with 2-6% dialkylated products and 14-35% recovered starting material.

(6) BOP, (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate: (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Peptide Coupling Reactions IV—Benzotriazolyl *N*-Oxytrisdimethylamino Phosphonium Hexafluorophosphate (B.O.P.) *Tetrahedron Lett.* **1975**, 1219-1222. (b) Hudson, D. Methodological Implications of Simultaneous Solid-Phase Peptide Synthesis. 1. Comparison of Different Coupling Procedures. *J. Org. Chem.* **1988**, *53*, 617-624.

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Table I. Opioid Receptor Binding Affinity of [D-Pro[10]]Dyn A-(1-11)[a]

peptide	K_i (nM) κ	μ	δ	K_i ratio, κ/μ/δ
[D-Pro[10]]Dyn A-(1-11)	0.030 ± 0.001	0.24 ± 0.002	2.1 ± 0.4	1/8/70
N,N-diallyl analogue	3.6 ± 0.5	31.7 ± 3.8	149 ± 58	1/8.8/41
N-allyl analogue	0.049 ± 0.001	10.9 ± 0.5	449 ± 15	1/222/9160
N-CPM analogue	0.020 ± 0.001	9.6 ± 0.6	558 ± 8.9	1/480/27900
N-benzyl analogue	0.029 ± 0.001	31.1 ± 0.2	176 ± 17	1/1070/6080
U-50,488[b]	1.16 ± 0.08	206 ± 3		1/178
U-69,593[b]	1.06 ± 0.02	551 ± 45		1/520

[a] The inhibitory effects of [D-Pro[10]]Dyn A-(1-11) and its analogues on the binding of [^{3}H]bremazocine (κ) in guinea pig cerebellum membranes and of [^{3}H]DAMGO (μ) and [^{3}H]DPDPE (δ) in rat forebrain were determined as previously described (ref 7), except that 100 nM DAMGO was included in the [^{3}H]bremazocine binding assay. The binding assays for the peptides were carried out at 4 °C in the presence of peptidase inhibitors. Nonspecific binding of [^{3}H]bremazocine, [^{3}H]DAMGO, and [^{3}H]DPDPE was determined in the presence of 1 μM Dyn A-(1-13) amide, 10 μM levorphanol, and 10 μM unlabeled DPDPE, respectively. Equilibrium inhibition constants (K_i) were calculated from the Cheng and Prusoff's equation (ref 15), using 0.0549, 0.314, and 7.63 nM for the K_D values of tritiated bremazocine, DAMGO, and DPDPE, respectively. [b] Binding assays for U-50,488 and U-69,593 were performed at 25 °C.

Table II. Opioid Activity of [D-Pro[10]]Dyn A-(1-11) Analogues in the Guinea Pig Ileum[a]

peptide	IC_{50} (nM)	naloxone pA_2[b]
[D-Pro[10]]Dyn A-(1-11)	0.22 (0.11-0.49)	7.2 (6.6-7.7)
N,N-diallyl analogue	687 (210-2250)[c]	
N-allyl analogue	18.3 (13.2-22.4)	7.6 (7.0-8.2)
N-CPM analogue	2.16 (1.6-2.8)	7.3 (6.8-7.7)
N-benzyl analogue	990 (657-1500)	
U-50,488	2.61 (2.22-3.07)	7.0 (5.8-8.2)

[a] The guinea pig ileum assays were performed as previously described (ref 7). Ninety-five percent confidence intervals are given in parentheses. [b] pA_2 values were determined by the method described in ref 9. The pA_2 value for morphine was 8.2 (7.9-8.5). [c] Shallow dose-response curve with a maximum response of 65%.

In conclusion, several N-monoalkylated [D-Pro[10]]Dyn A-(1-11) derivatives have been prepared which are highly selective for κ-opioid receptors. These peptides are the most κ-selective opioid peptides reported to date. Their κ-receptor selectivities are equal to or greater than those of the κ-selective nonpeptide agonists U-50,488[13] and U-69,593,[14] and their affinities for κ-receptors are greater (see Table I). Thus these peptides should be useful tools in further studies of the structure and functions of κ-opioid receptors. This work also shows that the low κ-receptor affinity and selectivity of the *N,N*-diallyl derivative result from the introduction of the second allyl substituent on the amine terminus.

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