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N-Alkylated derivatives of [D-Pro^{10}]dynorphin A-(1-11) are high affinity partial agonists at the cloned rat κ-opioid receptor

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Abstract

As part of an effort to develop peptides with selective κ-opioid antagonist activity, a series of *N*-alkylated [D-Pro^{10}]dynorphin A-(1-11) derivatives were made through solid-phase peptide synthesis: R-Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg-D-Pro–LysOH, where R = *N*-benzyl, *N*-cyclopropylmethyl, *N*,*N*-dicyclopropylmethyl, or *N*,*N*-diallyl. These derivatives and dynorphin A-(1-13)NH_2 were evaluated for κ-opioid receptor binding affinity and potency as inhibitors of adenylyl cyclase. Equilibrium competition binding experiments using [^{3}H]diprenorphine (≈ 600 pM) were performed on membranes prepared from cultured Chinese hamster ovary (CHO) cells stably expressing the rat κ-opioid receptor. Tissue prepared from this cell line was used to evaluate opioid peptide inhibition of forskolin-stimulated (50 μM) adenylyl cyclase activity. Displacement of [^{3}H]diprenorphine specific binding by these peptides was observed with a rank order of affinity (K_i, nM) = [D-Pro^{10}]dynorphin A-(1-11) (0.13) > dynorphin A-(1-13)NH_2 (0.34) > *N*-cyclopropylmethyl- (1.4) > *N*,*N*-dicyclopropylmethyl- (12.6) ≈ *N*-benzyl- (18.3) ≈ *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1–11) (26.0). A similar rank order was observed for potency of adenylyl cyclase inhibition (IC_{50}, nM): [D-Pro^{10}]dynorphin A-(1-11) (0.12) ≈ dynorphin A-(1-13)NH_2 (0.19) > *N*-cyclopropylmethyl- (2.7) > *N*,*N*-dicyclopropylmethyl- (13.2) ≈ *N*,*N*-diallyl- (18.0) ≈ *N*-benzyl-[D-Pro^{10}]dynorphin A-(1-11) (36.4). The peptides differed in their percent maximal inhibition of adenylyl cyclase activity: dynorphin A-(1-13)NH_2 (100%) ≈ *N*-cyclopropylmethyl- (94.3%) ≈ [D-Pro^{10}]dynorphin A-(1-11) (87.9%) > *N*-benzyl- (71.4%) ≫ *N*,*N*-dicyclopropylmethyl- (23.6%) ≈ *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) (18.9%). As the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives were found to have only weak partial agonist activity with respect to adenylyl cyclase inhibition, they were evaluated for their ability to reverse dynorphin A-(1-13)NH_2 (10 nM) inhibition of adenylyl cyclase activity. *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) reversed dynorphin A-(1-13)NH_2 inhibition to levels equal to the maximal inhibition produced by *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) alone. This weak partial agonism combined with nanomolar potency render the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) compounds promising leads for further attempts to synthesize peptide κ-opioid receptor antagonists. © 1997 Elsevier Science B.V.

Keywords: Partial agonism; Opioid; Dynorphin

1. Introduction

The analgesic properties of opium and its derivatives have been recognized for centuries. Despite significant side effects including psychoactivity, respiratory depression, nausea and risk of tolerance and dependence, opioid drugs remain among the most useful for the treatment of pain. Progress in understanding the molecular mechanisms underlying the action of opioids may allow development of less toxic opioid analgesics.

Pharmacological evaluation of opioid activity indicates the presence of at least three distinct receptor types, termed δ, κ and μ (reviewed by Knapp et al., 1995). Endogenous peptide ligands for these receptors have been identified and include the endorphins, dynorphins and enkephalins. Activation of opioid receptors by these endogenous peptides and other opioid agonists results in activation of the inhibitory G-protein, G_i (Hsia et al., 1984). This G-protein

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subtype effects a reduction in adenylyl cyclase activity, lowering intracellular concentrations of cyclic AMP (Blume et al., 1979).

Multiple receptor subtypes have been postulated for each of the primary δ-, κ- and μ-opioid receptor types (reviewed by Webster et al., 1993). This array of opioid receptors and their overlapping patterns of distribution in tissues have made determining the selectivity of opioid ligands for individual receptor subtypes difficult. Overlapping receptor distribution has also produced a lack of receptor-specific models suitable for investigating the efficacy of opioids in altering cellular effector systems. These problems have been remedied by the recent production of cDNA clones for δ-, κ- and μ-opioid receptors.

Expression of cloned opioid receptors in mammalian cell lines, and use of resulting tissue in radioligand binding assays, has been useful in clearly determining the relative affinities of an array of opioid ligands for each primary receptor type (Raynor et al., 1994). More significantly, these cell lines are also easily employed in functional assays, allowing accurate determination of opioid effects mediated through different receptor subtypes. This type of subtype-specific functional assay is a significant improvement over conventional bioassays which employ tissues that express multiple opioid receptor subtypes (e.g., assays based on guinea-pig ileum and mouse vas deferens). In the work reported here we have used a Chinese hamster ovary (CHO) cell line, stably expressing the rat κ-opioid receptor, to demonstrate that derivatives of the κ-selective peptide agonist [D-Pro^{10}]dynorphin A-(1-11) (Gairin et al., 1985) are high-affinity κ-opioid receptor partial agonists. These partial agonists represent progress toward development of κ-specific peptide antagonists.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Boehringer Mannheim, GIBCO BRL, ICN, or Sigma. [^{3}H]Adenine (26.9 Ci/mmol) and [^{14}C]cyclic AMP (52.3 mCi/mmol) were purchased from Du Pont/NEN (Boston, MA, USA). [^{3}H]Diprenorphine (31 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Bestatin, captopril and forskolin were purchased from Sigma (St. Louis, MO, USA). Bio-Rad AG-50W-X4 cation exchange resin was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Dynorphin A-(1-13)NH_2 and [D-Pro^{10}]dynorphin A-(1-11) were purchased from Peninsula Laboratories (Belmont, CA, USA). L-Leucyl-L-leucine was purchased from Bachem Bioscience (Philadelphia, PA, USA). Nor-binaltorphimine was purchased from Research Biochemicals International (Natik, MA, USA). 4-[(3-Butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone (Ro 20-1724) was a gift from Hoffmann-La Roche (Nutley, NJ, USA).

2.2. Peptide synthesis

N-alkylated [D-Pro^{10}]dynorphin A-(1-11) derivatives, R_1R_2Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg-D-Pro–LysOH, were prepared by solid phase peptide synthesis as described elsewhere (Choi et al., 1992; Choi et al., data not shown) using 9-fluorenylmethoxycarbonyl-protected amino acids. The *N*-alkylated tyrosine derivatives incorporated at the *N*-terminus were prepared by reaction of tyrosine *t*-butyl ester with the appropriate alkyl halide, followed by removal of the *t*-butyl group with trifluoroacetic acid. The peptides examined in the studies described below were *N*-cyclopropylmethyl-, *N*,*N*-dicyclopropylmethyl-, *N*-benzyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11).

2.3. CHO cell cultures

The CHO cell line expressing the rat κ-opioid receptor used in these experiments was a gift from Dr. David Grandy (Vollum Institute, Oregon Health Sciences University, Portland, OR, USA). This cell line was created as described by Bunzow et al., 1995. Cells were grown in F12 Ham's media supplemented with 10% fetal bovine serum, in 5% CO_2, at 37°C, to approximately 90% confluency. For adenylyl cyclase assays, cells were cultured in 35 mm six-well plates. For equilibrium binding assays, cells were scraped from 100 mm culture dishes using a rubber policeman and homogenized in ice-cold 25 mM Tris buffer (pH 7.2) using a Dounce homogenizer (A pestle), and centrifuged at $37\,000 \times g$ for 10 min. The resulting total particulate fraction was washed by resuspending, homogenizing, and centrifuging as described above three additional times. Final pellets were resuspended in ice-cold 25 mM Tris buffer (pH 7.2) immediately before use. Protein concentrations were determined according to the method described by Lowry et al., 1951.

2.4. Equilibrium competition binding assays

Equilibrium competition binding experiments were performed at 25° for 90 min in 25 mM Tris, 3 mM Mg^{2+} acetate buffer (pH 7.2) in a final volume of 2 ml containing κ-opioid receptor-expressing CHO cell membranes (100–180 μg protein), [^{3}H]diprenorphine (540–650 pM), various concentrations of opioid competitors, and peptidase inhibitors (10 μM bestatin, 30 μM captopril, 50 μM L-leucyl-L-leucine). Reactions were terminated by rapid filtration over GF/B filters (presoaked in 0.5% polyethylenimine to reduce nonspecific binding) using a Brandel cell harvester. Filters were transferred to vials and agitated overnight in a scintillation cocktail. Amounts of filter-trapped radioactivity were determined using a Beckman LS 6000SC scintillation counter.

2.5. Adenylyl cyclase assays

Adenylyl cyclase assays were performed according to a modified version of the method described by Salomon,

1979. Kappa-opioid receptor-expressing CHO cell cultures were washed twice with serum-free F12 Ham's media and then incubated for 4 h in 1 ml of the same media containing 1.2 μCi [^{3}H]adenine. The tritium-containing media was then aspirated and replaced with serum-free F12 Ham's media containing a phosphodiesterase inhibitor (50 μM Ro 20-1724) and peptidase inhibitors (10 μM bestatin, 30 μM captopril, 50 μM L-leucyl-L-leucine). These cultures were incubated at 37° for 40 min in the presence of 50 μM forskolin and various concentrations of opioids. Incubations were terminated by the addition of 300 μl of Stop Solution (2% SDS, 1.3 mM cyclic AMP), followed by addition of 100 μl concentrated perchloric acid, and 750 μl water. [^{14}C]cyclic AMP (5000 cpm in 50 μl) was added to each plate to correct for recovery. After transferring the contents of culture dishes to 1.5 ml centrifuge tubes, 12 M KOH was added to neutralize the samples. The resulting precipitate was pelleted by centrifuging at $10\,000 \times g$ for 10 min. Cyclic AMP in the supernatants was isolated by sequential chromatography over Bio-Rad AG-50W-X4 cation exchange resin and neutral alumina. Concentrations of [^{3}H]cyclic AMP and [^{14}C]cyclic AMP in eluates were determined simultaneously using a Beckman LS 6000SC scintillation counter (^{3}H channels 0–250, ^{14}C channels 350–670). Counts were corrected for crossover and recovery.

2.6. Data analysis

Data generated in equilibrium binding experiments and adenylyl cyclase assays were analyzed by fitting a logistic equation to data points using GraphPad Prism software. The equation used was:

$$\text{Bound} = \min + \frac{(\max - \min)}{1 + \left(10^{[L]}/10^{IC_{50}}\right)}, \quad (1)$$

where max represents binding or incorporation in the absence of competitor, min represents binding or incorporation in the presence of an excess concentration of competitor, [L] represents the concentration of competitor, and IC_{50} represents the [L] that will produce a 50% percent inhibition of binding. For analysis of specific binding in equilibrium competition binding experiments, min and max were fixed at 0% and 100%, respectively. Inhibitory binding constants (K_i) were calculated from IC_{50} values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973, diprenorphine $K_d = 0.45$ nM). Fits of Eq. (1) were compared to fits incorporating a slope factor for adenylyl cyclase data or that of a two-site model for binding data using the F statistic. In each case the fit of the more complicated model was not significantly better than that described by Eq. (1).

3. Results

3.1. Displacement of [^{3}H]diprenorphine binding

The ability of dynorphin A-(1-13)NH_2 and our series of [D-Pro^{10}]dynorphin A-(1-11) derivatives to inhibit the specific binding of [^{3}H]diprenorphine to κ-opioid receptor-expressing CHO membranes was evaluated in competition binding experiments. Results of these experiments demon-

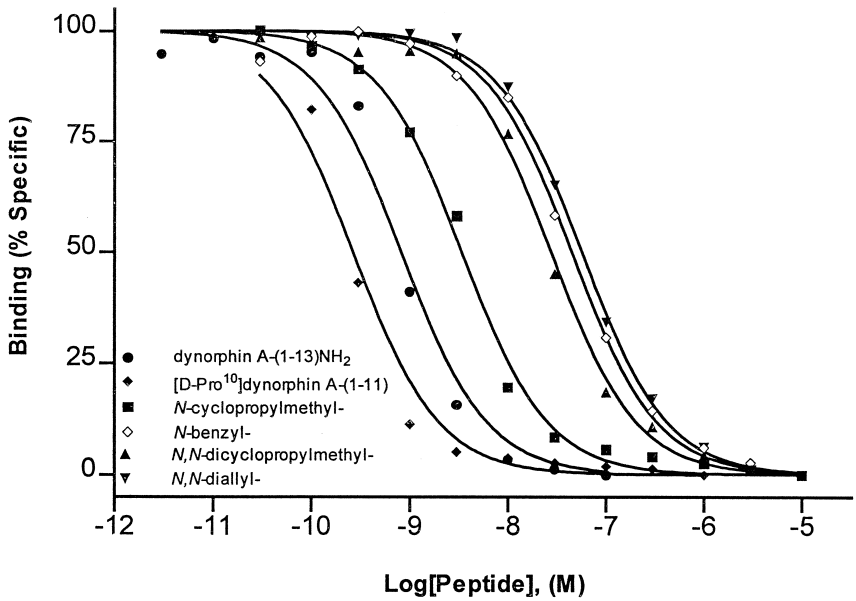

Fig. 1. Dynorphin A-(1-13)NH_2, [D-Pro^{10}]dynorphin A-(1-11), and [D-Pro^{10}]dynorphin A-(1-11) derivatives inhibiting the specific binding of [^{3}H]diprenorphine (540–650 pM). Membranes (100–180 μg of protein) prepared from CHO cells stably expressing the rat κ-opioid receptor were incubated at 25°C for 90 min. [D-Pro^{10}]dynorphin A-(1-11) derivatives are labeled with their *N*-alkyl modification. Shown are representative experiments performed in triplicate.

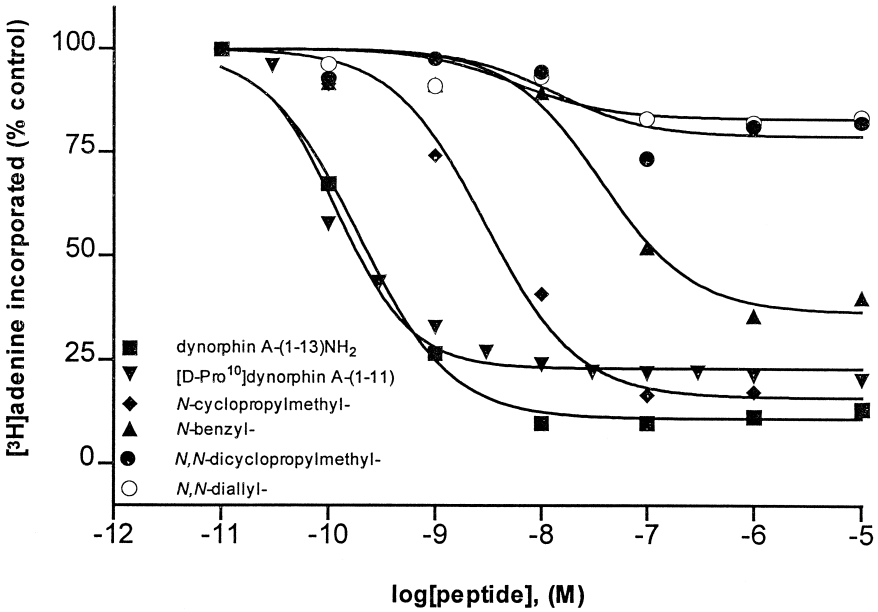

Fig. 2. Inhibition of forskolin-stimulated [^{3}H]adenine incorporation to cyclic AMP. CHO cells, stably expressing the rat κ-opioid receptor, were treated for 20 min with 50 μM forskolin (100% of control) and various concentrations of dynorphin A-(1-13)NH_2, [D-Pro^{10}]dynorphin A-(1-11), and [D-Pro^{10}]dynorphin A-(1-11) derivatives (labeled according to their *N*-alkyl modification). Data points represent means of one to three experiments performed in triplicate.

strated that each peptide displaced [^{3}H]diprenorphine from an apparently single binding site with high affinity (Fig. 1). Rank order of inhibition was (K_i, nM) = [D-Pro^{10}]dynorphin A-(1-11) (0.13) > dynorphin A-(1-13)NH_2 (0.34) > *N*-cyclopropylmethyl- (1.4) > *N,N*-dicyclopropylmethyl- (12.6) ≈ *N*-benzyl- (18.3) ≈ *N,N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) (26.0). These results are consistent with the specific binding of dynorphin A-(1-13)NH_2 and the [D-Pro^{10}]dynorphin A-(1-11)NH_2 derivatives to κ-opioid receptors.

3.2. Inhibition of adenylyl cyclase activity

The functional consequences of receptor binding by the peptides were investigated by measuring forskolin-stimulated adenylyl cyclase activity in κ-opioid receptor-expressing CHO cell cultures. Inasmuch as [D-Pro^{10}]dynorphin A-(1-11) demonstrated agonist activity similar to that of dynorphin A-(1-13)NH_2 in guinea-pig ileum and sodium-shift assays (Choi et al., data not shown), and in the adenylyl cyclase assays reported here, the latter was used as a reference compound in our experiments. Results demonstrated that each peptide inhibited adenylyl cyclase activity in a concentration-dependent manner (Fig. 2). The rank order of adenylyl cyclase inhibition was similar to that observed for displacement of specific [^{3}H]diprenorphine binding (IC_{50}, nM) = [D-Pro^{10}]dynorphin A-(1-11) (0.12) ≈ dynorphin A-(1-13)NH_2 (0.19) > *N*-cyclopropylmethyl- (2.7) > *N,N*-dicyclopropylmethyl- (13.2) ≈ *N,N*-diallyl- (18.0) ≈ *N*-benzyl-[D-Pro^{10}]dynorphin A-(1-11) (36.4).

The most notable feature of the adenylyl cyclase inhibition was distinct differences in the maximal effect of the

Table 1
Dynorphin A-(1-13)NH_2 and [D-Pro^{10}]dynorphin A-(1-11) derivative binding affinities and potencies as inhibitors of adenylyl cyclase in κ-opioid receptor-expressing CHO cells

Peptide	[^{3}H]diprenorphine K_i (nM)[a]	Adenylyl cyclase IC_{50} (nM)[b]	Efficacy (%)[c]
Dynorphin A-(1-13)NH_2	0.34 (0.27–0.41)	0.19 (0.14–0.26)	100.0
[D-Pro^{10}]dynorphin A-(1-11)	0.13 (0.01–0.16)	0.12 (0.08–0.16)	87.9
N-cyclopropylmethyl-[D-Pro^{10}]dynorphin A-(1-11)	1.4 (1.24–1.56)	2.7 (1.7–4.4)	94.3
N,N-dicyclopropylmethyl-[D-Pro^{10}]dynorphin A-(1-11)	12.6 (11.2–14.2)	13.2 (0.87–200.2)	23.6
N-benzyl-[D-Pro^{10}]dynorphin A-(1-11)	18.3 (16.2–20.7)	36.4 (13.8–96.5)	71.4
N,N-diallyl-[D-Pro^{10}]dynorphin A-(1-11)	26.0 (24.4–27.7)	18.0 (0.95–339.7)	18.9

[a]Mean K_i values determined from equilibrium competition binding using [^{3}H]diprenorphine. Values in parentheses represent 95% confidence interval.
[b]Mean IC_{50} values as inhibitors of adenylyl cyclase.
[c]% Maximum inhibition of adenylyl cyclase.

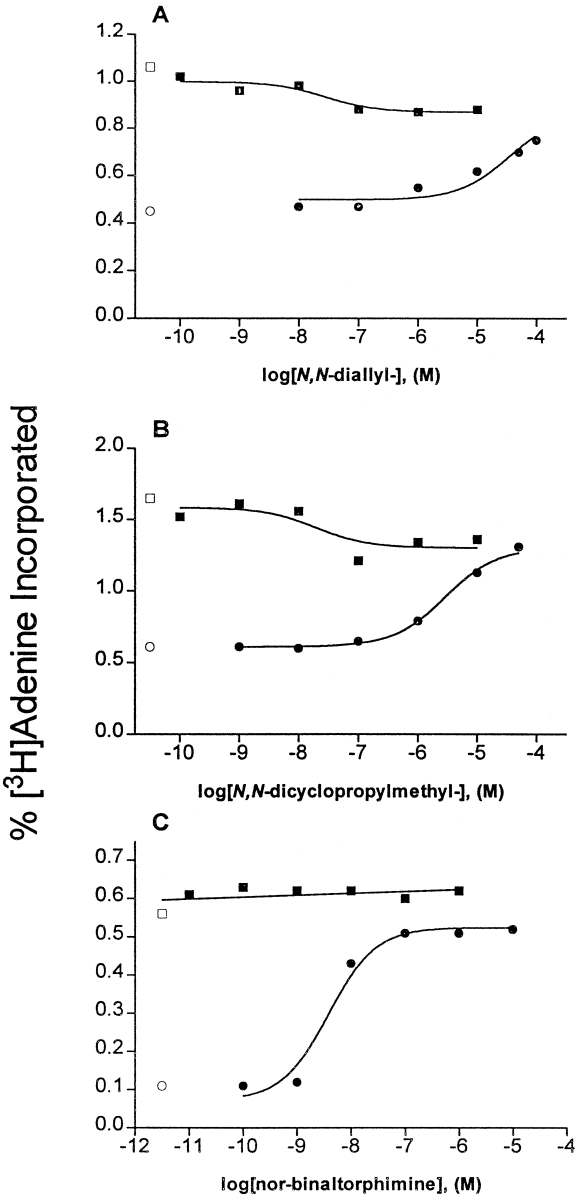

Fig. 3. Reversal of dynorphin A-(1-13)NH_3-mediated inhibition of forskolin-stimulated [^{3}H]adenine incorporation to cyclic AMP. CHO cells, stably expressing the rat κ-opioid receptor, were treated for 20 min with 50 μM forskolin and various concentrations of nor-binaltorphimine, *N*,*N*-diallyl-, and *N*,*N*-dicyclopropylmethyl-[D-Pro^{10}]dynorphin A-(1-11) with (●) or without (■) 10 nM dynorphin A-(1-13)NH_2. Open symbols indicate 50 μM forskolin (□) and 10 nM dynorphin A-(1-13)NH_2 + 50 μM forskolin (○) controls. Shown are representative experiments performed in triplicate.

peptides. Using the values obtained for dynorphin A-(1-13)NH_2 (10 nM) as a reference (100%), the synthetic opioid peptides displayed a range of efficacies. Treatment of cultures with the *N*-benzyl-, *N*,*N*-dicyclopropylmethyl-, and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives resulted in only a partial inhibition of adenylyl cyclase activity relative to the effect of the full agonist, dynorphin A-(1-13)NH_2. Of these partial agonists, the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives produced particularly modest inhibition of adenylyl cyclase activity. This modest inhibition is consistent with a lack of efficacy in guinea-pig ileum assays and low sodium-shift values obtained in [^{3}H]diprenorphine competition binding experiments (Choi et al., data not shown). The peptide affinities for the rat κ-opioid receptor, as well as potencies and efficacies as inhibitors of adenylyl cyclase activity are compared in Table 1.

3.3. Antagonism of dynorphin A-(1-13)NH_2-mediated inhibition of adenylyl cyclase activity

To further characterize the partial agonism produced by *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives, their ability to antagonize the inhibition of adenylyl cyclase produced by a fully-effective concentration of dynorphin A-(1-13)NH_2 (10 nM) was investigated. Results of these antagonism experiments showed that the partial agonists effectively reversed dynorphin A-(1-13)NH_2-mediated inhibition (Fig. 3). In contrast to the complete reversal (to control levels) produced by the pure κ-opioid antagonist nor-binaltorphimine, the peptide partial agonists produced an incomplete reversal of dynorphin A-(1-13)NH_2-mediated inhibition of adenylyl cyclase. This incomplete reversal plateaued at a level similar to that produced by maximally-effective concentrations of the partial agonists alone, an effect expected for partial agonists.

4. Discussion

In the studies presented herein, cultures and membranes prepared from CHO cells stably expressing the rat κ-opioid receptor were used to assess κ-opioid-mediated activity of dynorphin A-(1-13)NH_2 and [D-Pro^{10}]dynorphin A-(1-11) derivatives. Expression of opioid receptors was confirmed by the ability to inhibit specific [^{3}H]diprenorphine binding with κ-selective opioids. The ability of dynorphin A-(1-13)NH_2 and [D-Pro^{10}]dynorphin A-(1-11) derivatives to attenuate adenylyl cyclase activity in CHO cells indicated that expression of the κ-opioid receptor results in a functional regulation of this enzyme. The inhibitory nature of this regulation is consistent with that seen in endogenous systems mediated by the inhibitory G-protein, G_i (Konkoy and Childers, 1989). Use of κ-opioid receptor-expressing CHO cells allowed us to demonstrate that *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) bind to the rat κ-opioid receptor with high affinity, but retain only partial efficacy for inhibition of adenylyl cyclase activity.

The clinical utility of opioids with partial efficacy has been well-documented (reviewed by Hoskin and Hanks, 1991). Drug design programs have produced partial agonists (e.g., pentazocine, butorphanol, nalbuphine) that are effective analgesics, with side-effect profiles more favorable than morphine. Many of these programs have targeted

agonism of the κ-opioid receptor, due to a possible reduced liability for tolerance and dependence of κ-agonists relative to δ and μ (Gmerek et al., 1987). Selective partial agonism of opioid receptor subtypes may allow an analgesic response to be produced at dosages lower than those required to produce adverse effects. Our goal in the design of the peptides tested here was to synthesize κ-receptor selective peptide antagonists for use as tools in the pharmacological evaluation of such novel κ-opioids.

The partial agonism of the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives that were synthesized during this project was not anticipated. In isolated tissue preparations (guinea-pig ileum and rabbit vas deferens) the *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivative appears to act as an antagonist at κ-receptors (Gairin et al., 1988). Our ability to demonstrate that this peptide acts as a partial agonist may be attributed to the use of the κ-opioid receptor-expressing CHO cell line. Unlike smooth muscle preparations, this source of adenylyl cyclase-coupled κ-opioid receptors is free from contamination by other opioid receptor types. In addition, the cloned receptor is expressed at high levels ($\approx$ 900 fmol/mg protein, data not shown). High-level expression results in amplification of receptor-mediated signaling, rendering the detection of compounds with low efficacies more sensitive.

The molecular mechanisms underlying opioid-mediated partial efficacy are not well understood. Partial agonism of a G-protein-coupled β-adrenergic receptor has been more thoroughly investigated (reviewed by Jasper and Insel, 1992). In this β-adrenergic system, binding of a ligand to the receptor involves multiple interactions between portions of the ligand and specific amino acids in the receptor. Amino acids that are critical for the effective association of a ligand–receptor complex with adenylyl cyclase have been identified (Strader et al., 1989). Partial agonists may lack the ability to participate in some of these interactions, or may interact with different facility, resulting in a receptor–ligand complex that is conformationally distinct from that produced by full agonists (Vauquelin and Maguire, 1980). The functional consequences of conformational differences may be related to changes in the ligand–receptor complex–G-protein interaction. This type of mechanism was demonstrated in a reconstituted muscarinic cholinergic receptor system (Tota and Schimerlik, 1990) where the reduced efficacy of partial agonists to inhibit adenylyl cyclase is related to a reduced affinity of the partial agonist–receptor complex for the G-protein. This reduction in affinity for the G-protein leads to lower steady-state concentrations of activated G-protein and, ultimately, to reduced effect. Similar mechanisms may be involved in the opioid receptor-mediated partial agonism produced by the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) derivatives.

Some insight to the mechanism of κ-opioid partial agonists may be gained by considering work done with κ-specific opioid antagonists. The prototypic κ-specific antagonist, nor-binaltorphimine (Portoghese et al., 1987), is a bivalent ligand, consisting of two naltrexone-derived pharmacophores. Structure–activity studies of nor-binaltorphimine and congeners suggest that these compounds interact with at least two recognition sites within the κ-opioid receptor (reviewed by Portoghese, 1989). These recognition sites have been proposed to play separate roles in mediating subtype selectivity and efficacy (Portoghese, 1989). In the case of nor-binaltorphimine, structure–activity studies suggest the importance of the N17′ basic nitrogen for κ-selectivity (Portoghese et al., 1994). This basic nitrogen has been proposed to mimic dynorphin's basic arginine side chain at position 7. This basic residue, which is present in our dynorphin derivatives, has been implicated in the peptides' κ-selectivity (Chavkin and Goldstein, 1981), although more recently the importance of this position for κ-selectivity has been questioned (Kawasaki et al., 1993). Structure–activity studies have shed less light on the issue of efficacy. The diminished efficacy of the *N*,*N*-dicyclopropylmethyl- and *N*,*N*-diallyl-[D-Pro^{10}]dynorphin A-(1-11) partial agonists may be due to the *N*-terminal dialkyl modifications, as these are the portions that differ from the parent full agonist, [D-Pro^{10}]dynorphin A-(1-11).

Interpretation of opioid activity based on structure–activity studies has been extended by identification of residues important for nor-binaltorphimine interaction with chimeric opioid receptors (Hjorth et al., 1995). Substitution of glutamic acid with lysine at position 297 (at the interface of the sixth transmembrane-spanning-domain and the third extracellular loop) of the κ-opioid receptor significantly reduces its affinity for the κ-selective antagonist. The authors suggest that Glu^{297} is important for interaction with the N17′ basic nitrogen group of of nor-binaltorphimine. Presumably this region of the receptor is important for this compound's κ-selectivity. As is the case with the structure–activity studies, identification of receptor regions important for mediating the efficacy of opioid ligands have not been made. Perhaps partially-efficacious compounds such as the [D-Pro^{10}]dynorphin A-(1-11) derivatives described herein will be useful tools for the study of the molecular basis of opioid efficacy.

Overall, the synthesis of these κ-opioid partial agonists, and our ability to clearly measure their efficacy represents progress toward development of peptides that will antagonize effects mediated by the κ-opioid receptor. The use of heterologous expression systems with individual opioid receptor subtypes will be central to our screening of additional peptides for κ-specific antagonism.

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