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Communications to the Editor

A Novel Acetylated Analogue of Dynorphin A-(1−11) Amide as a κ-Opioid Receptor Antagonist

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Narcotic analgesics such as morphine are a mainstay for treating severe pain, but these μ-opioid analgesics are associated with severe side effects, most importantly addiction liability and respiratory depression. Therefore there is considerable interest in exploring compounds that interact with other opioid receptors as potential analgesics without these serious side effects. κ-Opioid receptors are present in human brain and spinal cord in high concentrations,[1−3] and κ-selective compounds (see ref 4 for a review) and a dynorphin analogue E-2078[5,6] have been studied as potential analgesics. There is also considerable interest in κ-selective compounds as potential neuroprotective and anticonvulsant agents,[7] and recently it was discovered that κ-opioid agonists can downregulate human immunodeficiency virus (HIV-1) expression in human microglial cells, suggesting that such compounds could be useful in the treatment of HIV-1-associated encephalopathy.[8] Therefore a better understanding of how κ receptors function at the molecular level could be very important in the development of new therapeutic agents.

Dynorphin A (Dyn A), a heptadecapeptide isolated from porcine pituitary, preferentially interacts with κ-opioid receptors and therefore is postulated to be an endogenous ligand for these receptors.[9,10] Dyn A shares a common N-terminal tetrapeptide "message" sequence with other mammalian opioid peptides and has a unique C-terminal "address" sequence which imparts high affinity for κ receptors.[11] The shortened peptides Dyn A-(1−13) and Dyn A-(1−11) account for most of the heptadecapeptide's biological activity and therefore are often used as the parent structures in structure−activity relationship studies of Dyn A.[10,11]

Selective antagonists are useful pharmacological tools in studying receptor function and the physiological and pharmacological actions of agonists. κ-Selective nonpeptide antagonists, most notably norbinaltorphimine (norBNI),[12] and selective peptide antagonists for μ and δ receptors (see ref 4) have been identified. The search for peptide antagonists for κ receptors, however, has met with limited success. Only a few Dyn A analogues with antagonist activity have been reported,[13−19] and these peptides generally exhibit weak antagonist activity and low selectivity for κ receptors. Our laboratory is interested in developing peptide-based antagonists for κ receptors in order to examine the differences in interactions between peptide agonists and antagonists with these receptors. Such compounds would also be complimentary pharmacological tools to norBNI to study the involvement of Dyn A and κ receptors in various physiological and pharmacological functions. The examination of Dyn A analogues is a promising approach to identify derivatives with reduced efficacy as lead compounds in the search for peptide antagonists selective for κ receptors.

Recently, Orosz et al. reported that an N-terminal-protected tetrapeptide (Boc-Tyr-Lys-Trp-Trp-NH_2) derived from a pentapeptide found in Philippine cobra venom was a selective but weak κ receptor antagonist in the guinea pig ileum (GPI).[20] It antagonized ethylketocyclazocine (EKC) and DAMGO (Tyr-D-Ala-Gly-Me-Phe-$NH(CH_2)_2OH$), but only at very high concentrations (K_e = 5.4 and 117 μM, respectively). We postulated that this novel tetrapeptide sequence may be equivalent to the "message" sequence of Dyn A because it retains two aromatic residues at positions 1 and 4, which are critical structural features for the opioid activity of Dyn A.[21] Since this tetrapeptide exhibited antagonist activity at κ receptors, we applied the "message−address" concept[11]

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JVA-901 Ac-Tyr-Lys-Trp-Trp-Leu-Arg-Arg-D-Ala-Arg-Pro-Lys-NH_2

Tetrapeptide Ac-Tyr-Lys-Trp-Trp-NH_2

[D-Ala^8]Dyn A-(1–11)NH_2 Tyr-Gly-Gly-Phe-Leu-Arg-Arg-D-Ala-Arg-Pro-Lys-NH_2

Figure 1. Peptide sequences indicating proposed "message" (—) and "address" (- - -) sequences.

to design compounds with enhanced κ receptor affinity and antagonist potency. Previously we have successfully applied this concept to convert μ and δ peptide antagonists into chimeric Dyn A analogues with enhanced κ receptor affinity and reduced efficacy.[22,23] Thus we prepared a chimeric peptide, JVA-901, by combining the N-terminal-acetylated derivative of the tetrapeptide described by Orosz et al.[20] with the "address" sequence (residues 5−11) of [D-Ala^8]Dyn A-(1−11)NH_2 (Figure 1). We also synthesized the acetylated derivative of Orosz's tetrapeptide for comparison.

The peptides were prepared by solid-phase peptide synthesis on a PAL-PEG (peptide amide linker-poly(ethylene glycol)) resin (PerSeptive Biosystems Inc., Framingham, MA) using Fmoc-amino acids[24] and were acetylated with acetic anhydride. The peptides were cleaved from the support using Reagent B[25] and purified by preparative reversed-phase HPLC.[26]

The peptides were examined for their affinity for opioid receptors in radioligand binding assays using Chinese hamster ovary (CHO) cells expressing cloned opioid receptors (Table 1)[26] and for their efficacy in an adenylyl cyclase assay using the same cells.[27] Although the affinity of this initial lead peptide for κ receptors was substantially lower than that of [D-Ala^8]Dyn A-(1−11)NH_2, JVA-901 still displayed nanomolar affinity for κ-opioid receptors (Table 1). The tetrapeptide Ac-YKWW-NH_2 exhibited very low κ receptor affinity, consistent with the reported low-micromolar potency of the Boc-protected tetrapeptide in the GPI assay.[20] The "address" sequence alone (Ac[D-Ala^8]Dyn A-(5−11)NH_2) exhibited negligible affinity (K_i > 10 μM) for κ- as well as μ- and δ-opioid receptors (data not shown). Thus introduction of the "address" sequence of [D-Ala^8]Dyn A-(1−11)NH_2 to the acetylated tetrapeptide increased κ receptor affinity 68-fold. The μ-opioid receptor affinity of JVA-901 was also higher than that of the tetrapeptide, but the 14-fold increase was less than the increase observed at κ receptors. Thus the Dyn A "address" sequence enhanced the κ receptor selectivity of JVA-901 compared to the tetrapeptide by increasing κ receptor affinity more than μ receptor affinity; the selectivity of JVA-901 for κ receptors is similar to that of [D-Ala^8]Dyn A-(1−11)NH_2. The κ receptor selectivity of the acetylated tetrapeptide determined in these binding assays (K_i ratio (κ/μ) = 1/2.6) is considerably less than that reported for the Boc-protected tetrapeptide in functional assays in the GPI (K_e ratio (EKC/DAMGO) = 1/21.7).[20] The δ receptor affinity was very low for both JVA-901 and the acetylated tetrapeptide, with JVA-901 exhibiting slightly lower affinity than the tetrapeptide.

In the adenylyl cyclase assay in CHO cells expressing κ-opioid receptors, JVA-901 exhibited partial agonist activity with low efficacy (maximum of 28 ± 13% (n = 3)) of the maximum inhibition of cAMP formation observed for Dyn A-(1−13)NH_2. Because of its reduced efficacy, JVA-901 was examined for its ability to antagonize Dyn A-(1−13)NH_2 in this assay. This novel peptide reversed the agonist activity of Dyn A-(1−13)-NH_2 in a concentration-dependent manner (Figure 2); JVA-901 completely reversed the agonism of 1 nM Dyn A-(1−13)NH_2 (Figure 2b). These results for JVA-901 are consistent with the reported antagonist activity of the parent tetrapeptide.[20] Since the "message" sequence of JVA-901 has distinct structural differences from that of Dyn A, we are currently conducting further structure−activity relationship studies on the novel "message" sequence in JVA-901 to understand the antagonism exhibited by this compound and to enhance the affinity of this novel peptide for κ-opioid receptors.

In conclusion, a chimeric analogue of Dyn A, JVA-901, was designed using the "message−address" concept[11] by combining an acetylated derivative of the tetrapeptide reported by Orosz et al.[20] with the "address" sequence of [D-Ala^8]Dyn A-(1−11)NH_2. JVA-901 exhibited markedly enhanced κ receptor affinity and selectivity compared to the acetylated tetrapeptide. Moreover, this chimeric peptide demonstrated antagonist activity against Dyn A-(1−13)NH_2 in the adenylyl cyclase assay. These results reiterate the importance of the "address" sequence of Dyn A for κ receptor affinity and demonstrate the applicability of the "message−address" concept to designing new lead peptides with antagonist activity at κ-opioid receptors.

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Supporting Information Available: Characterization and amino acid analysis of JVA-901. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Opioid Receptor Binding Affinity of JVA-901 and the Acetylated Tetrapeptide[a]

peptide	K_i (nM ± SEM) κ	μ	δ	K_i ratio ($\kappa/\mu/\delta$)
JVA-901	19.8 ± 5.2	251 ± 22	5320 ± 1130	1/12.6/268
Ac-YKWW-NH_2	1367 ± 330	3590 ± 1140	3478 ± 410	1/2.6/2.54
[D-Ala[8]]Dyn A-(1−11)NH_2	0.19 ± 0.08	1.97 ± 0.05	12.2 ± 3.0	1/10.4/64.2

[a] [^{3}H]Diprenorphine, [^{3}H]DAMGO, and [^{3}H]DPDPE were used as the radioligands for κ, μ, and δ receptors, respectively. K_i values are the average ± SEM of 6−8 independent experiments for JVA-901; n = 2−3 for the tetrapeptide and 4−6 for [D-Ala[8]]Dyn A-(1−11)NH_2.

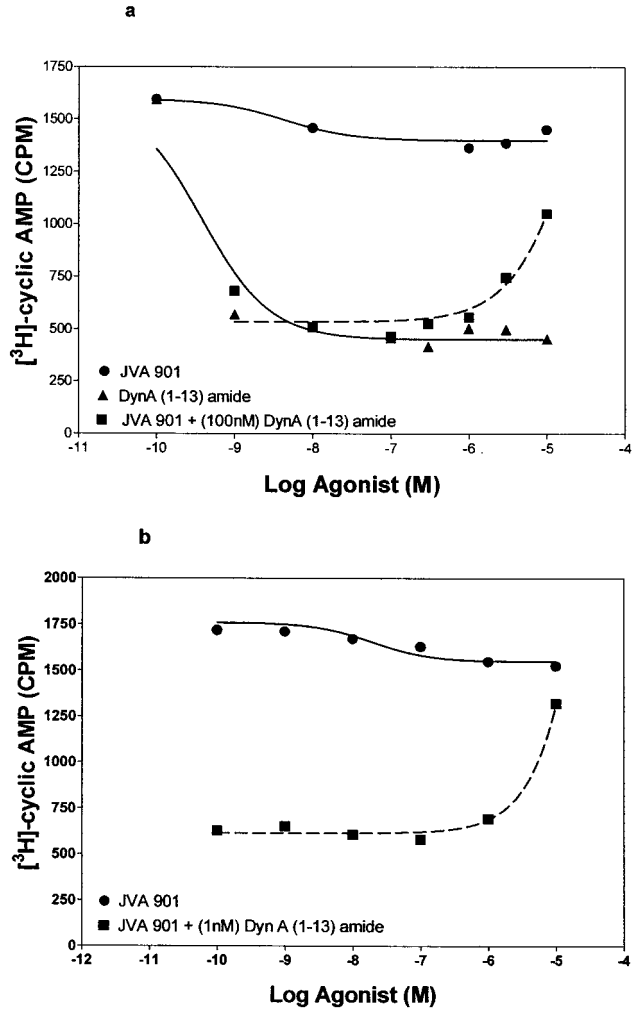

Figure 2. Inhibition of cAMP production by JVA-901 (●) and Dyn A-(1−13)NH_2 (▲) in CHO cells expressing κ-opioid receptors and reversal by JVA-901 (■) of the inhibition of cAMP production produced by (a) 100 nM and (b) 1 nM Dyn A-(1−13)NH_2. Data points were derived from a representative experiment performed in duplicate that was repeated three times. The mean IC_{50} values for JVA-901 and Dyn A-(1−13)-NH_2 as inhibitors of adenylyl cyclase were 9.1 ± 2.6 and 0.38 ± 0.2 nM, respectively.

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