Analogs of [D-Phe^4]CJ-15,208 with Modifications to Improve Kappa Opioid Receptor Antagonist Potency in the CNS as Potential Treatments for Substance Abuse

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Introduction

- We are exploring cyclic peptides with kappa opioid receptor (KOR) antagonist activity as potential treatments for relapse to substance abuse, specifically cocaine abuse.
  - Stress is a potent cause of relapse to drug-seeking behavior in abstinent individuals with substance use disorder. Stress exposure increases the levels of dynorphins, the endogenous KOR agonists.
  - KOR antagonists block KOR activation by the dynorphins and prevent stress-induced reinstatement of cocaine-seeking behavior in rodent models of cocaine use disorder (CUD).
- The macrocyclic tetrapeptide [D-Trp]CJ-15,208 is an orally active kappa opioid kappa opioid receptor (KOR) antagonist.[1]
  - Macrocyclic peptides are promising compounds for potential therapeutic development because they are stable to proteolytic degradation and can cross biological barriers.
  - Exploration of the structure-activity relationships (SAR) of [D-Trp]CJ-15,208 led to the identification of **[D-Phe^4]CJ-15,208** (*cyclo*[Phe^1-D-Pro-Phe-D-Phe^4]) as a 2nd-generation lead compound that is 6-fold more potent than [D-Trp]CJ-15,208 in a mouse model of CUD.[2]
  - Like [D-Trp]Cj-15,208 [D-Phe^4]CJ-15,208 undergoes rapid metabolism by human and mouse liver microsomes.[2]
- We are exploring the SAR of [D-Phe^4]CJ-15,208 to further improve its potency in the CNS after oral administration and improve its ADME properties.
  - Here we report the effects of amino acid substitution in position 1 on KOR affinity, metabolic stability and *in vivo* activity.

Methods

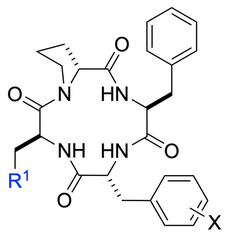
- **Synthesis.** Peptides were synthesized by a combination of solid phase peptide synthesis of the linear precursor peptides, followed by cyclization in solution as previously described and purified by normal phase flash chromatography plus reversed phase flash chromatography when needed.[2]
- **Metabolism**. The metabolic stability of the peptides (1 µM) was determined in human (HLM) and mouse liver microsomes (MLM; 1 mg/mL protein) by Curia Global, Buffalo, NY.
- ***In vitro* pharmacological evaluation.** KOR and mu opioid receptor (MOR) affinities and KOR antagonist activity in the cAMP assay were determined as previously described.[2] IC_{50} values represent antagonism of an IC_{80} concentration of U50,488 (10 nM) calculated from nonlinear regression analysis from 3-4 experiments, each performed in triplicate.
- ***In vivo* pharmacological testing.** Peptides were evaluated following oral (*per os*, p.o.) administration in a solution of 10%DMSO/10% Solutol/80% saline (0.9%) in the mouse 55 ºC warm water tail withdrawal (WWTW) assay as previously described.[2] KOR antagonism by the peptides was assessed against U50,488 administered either peripherally (10 mg/kg, i.p., -2.5 h) or centrally (30 nmol i.c.v. (intracerebroventricular), -2.5 h). To determine opioid receptor antagonist selectivity of the test peptides the mu or delta opioid receptor agonists morphine (10 mg/kg i.p.) and SNC-80 (100 nmol, i.c.v.) were administered in place of U50,488 and activity assessed.

  One peptide was evaluated for its ability to prevent reinstatement of stress-induced reinstatement of cocaine conditioned place preference (CPP) as previously described.[2]

  Statistical analysis as appropriate was performed with Prism 10.5 software by Student's t-test or one- or two-way ANOVA and further analyzed with appropriate post hoc tests.

Results

- We incorporated both aromatic and aliphatic amino acids in place of Phe^1 in [D-Phe^4]CJ-15,208 analog since Ala substitution of Phe^1 is tolerated in [D-Trp]CJ-15,208.[3]



Analogs of [D-Phe^4]CJ-15,208 with a substitution for Phe^1

- **KOR affinity, selectivity and antagonism**
  - Analogs containing an aromatic amino acid in position 1
    - Out of the more than 45 analogs >70% had a KOR affinity comparable to or higher than [D-Phe^4]Cj-15,208 ($K_i \leq 30$ nM) (Fig. 1)
    - KOR selectivity over mu opioid receptors (MOR) was generally modest (K_i (MOR)/K_i (KOR) $\leq$10)
      - Three analogs with high KOR affinity (K_i = 1-3 nM) exhibited much higher KOR selectivity (K_i ratio = 35-62) than [D-Phe^4]CJ-15,208 (K_i ratio = 11)
      - These are comparable or better than the KOR selectivity of the small molecule KOR antagonists aticaprant and navacaprant (19 and 40, respectively) that were in clinical trials for major depression
  - Analogs containing an aliphatic amino acid in position 1
    - 60% of the 18 analogs also have a KOR affinity comparable or higher than [D-Phe^4]Cj-15,208 (Fig. 1)
    - Seven analogs also exhibited high KOR selectivity (K_i ratio > 40)
  - KOR antagonist potency varied substantially. Most analogs were more potent than [D-Phe^4]CJ-15,208 (IC_{50} = 460 nM); half a dozen analogs were more potent than navacaprant (IC_{50} = 69 nM)

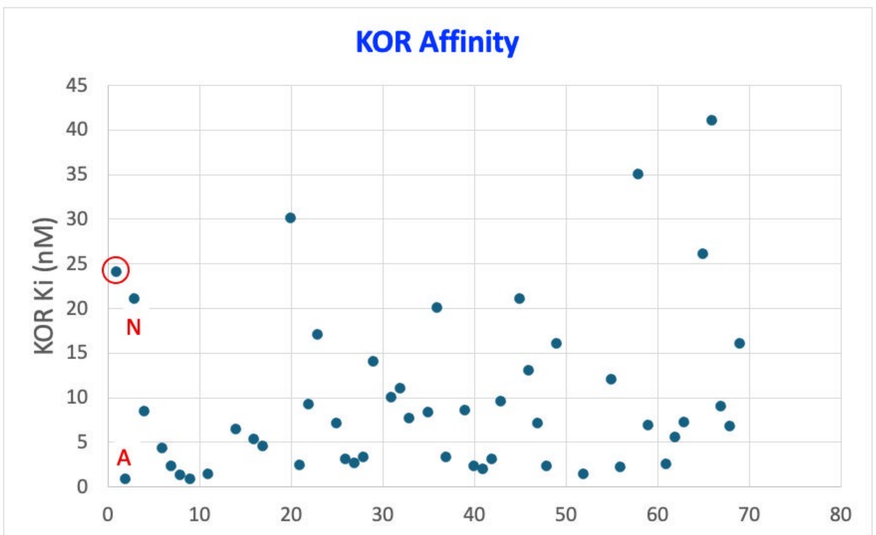


Fig. 1. KOR affinities of analogs containing an aromatic amino acid (peptides 4-49) or an aliphatic amino acid (peptides 52-69) in position 1 compared to [D-Phe^4]CJ-15,208 (circled) and the small molecule KOR antagonists aticaprant (A) and navacaprant (N). Analogs with K_i > 60 nM are not shown.

- **Metabolic stability**
  - [D-Phe^4]CJ-15,208 is metabolized rapidly by both human (HLM; $t_{1/2}$ = 4.2 min) and mouse liver microsomes (MLM; $t_{1/2}$ = 1.6 min)[2]
    - Seven analogs containing an aromatic amino acid in position 1 had half-lives in HLM of >20 minutes (Cl_{int} < 35 µg/min/mg protein)
    - Only one analog with an aliphatic amino acid in this position had a half-life in HLM >20 minutes
- ***In vivo* activity**
  - Analogs were screened after oral administration at 30 mg/kg, p.o.
    - None of the analogs tested exhibited substantial antinociception (agonist activity) in the 55º WWTW assay
  - Among the analogs containing an aromatic amino acid in position 1 screened to date
    - Seven out of 10 analogs screened antagonized i.p. U50,488
    - Two analogs antagonized centrally administered (i.c.v.) U50,488 (Fig. 2)

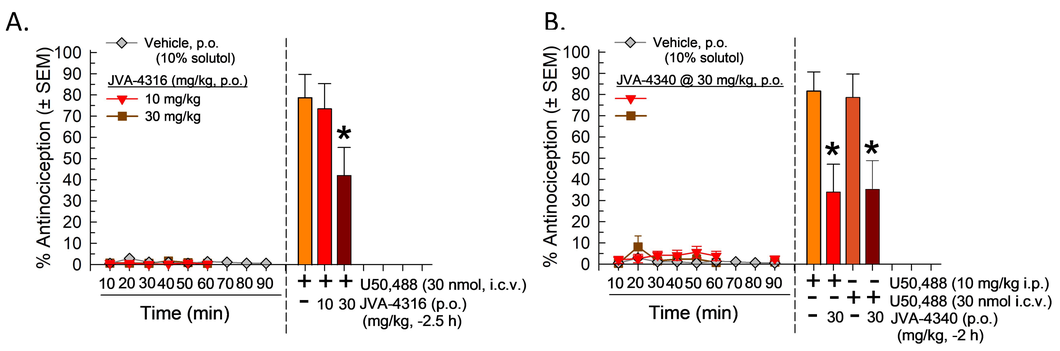


Fig. 2. Antagonism of U50,488 (30 nmol, i.c.v.) by A. JVA-4316 and B. JVA-4340. *p < 0.05 compared to vehicle treated mice

- Among the analogs containing an aliphatic amino acid screened to date
  - Three of the five analogs screened antagonized i.c.v. U50,488 (Fig. 3)

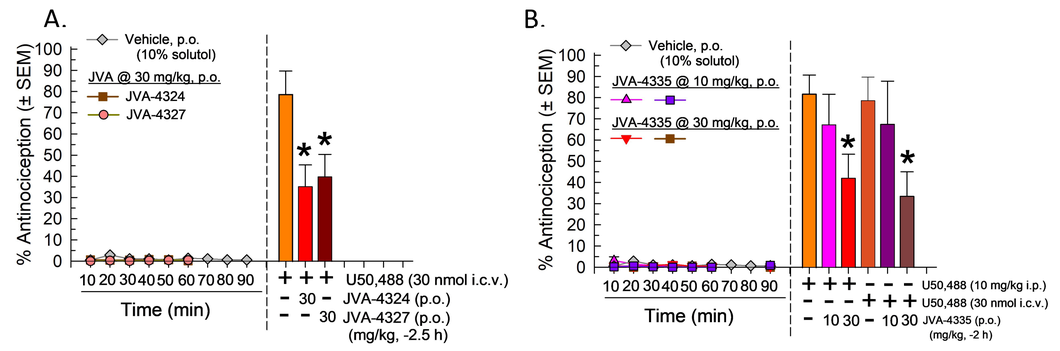


Fig. 3. Antagonism of U50,488 (30 nmol, i.c.v.) by A. JVA-4324, JVA-4327 and B. JVA-4335. *p < 0.05 compared to vehicle treated mice

- Analog JVA-4316
  - Selectively antagonized U50,488, but not the mu opioid receptor preferring agonist morphine or the delta opioid receptor selective agonist SNC-80
  - Antagonized U50,488 (i.p.) when administered 2.5 hours, but not 6 hours, prior to testing, similar to [D-Trp]- and [D-Phe^4]CJ-15,208.[1,2]
  - **Prevented stress-induced reinstatement of cocaine CPP at a dose of 10 mg/kg, p.o.** (Fig. 4A), which is comparable to [D-Phe^{4}CJ-15,208 [2] and navacaprant (Fig. 4B)

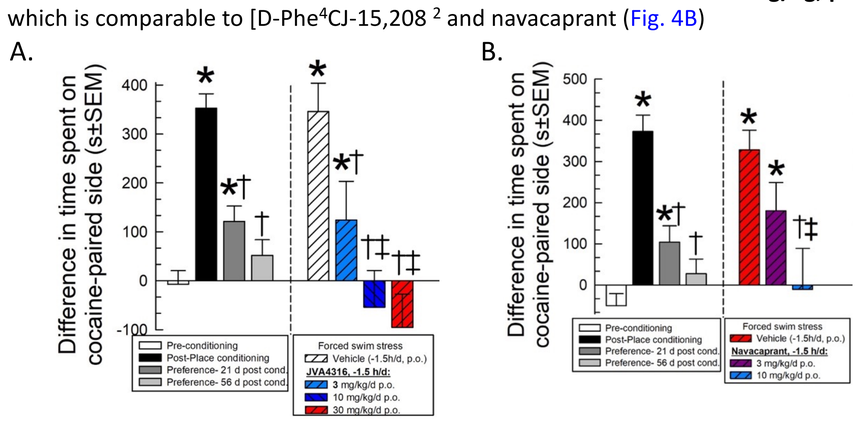


Fig. 4. A. JVA-4316 and B. the small molecule KOR antagonist navacaprant significantly decreased stress-induced reinstatement of cocaine CPP at a dose of 10, but not 3, mg/kg p.o. *, †, and ‡= sig. different (p < 0.05) from preconditioning, postconditioning and reinstated response, respectively

- Did not significantly decrease respiration, in contrast to morphine. It also did not result in hyperlocomotion (data not shown).

Conclusions

- A variety of aromatic and aliphatic amino acids are tolerated by KOR in position 1 of [D-Phe^4]Cj-15,208 analogs
  - Analogs with increased selectivity for KOR over MOR were identified
- The majority of the analogs underwent oxidative metabolism in liver microsomes with Cl_{int} > 35 µg/mL/mg protein
- Four analogs were identified that significantly antagonized centrally administered (i.c.v..) U50,488, consistent with central KOR antagonism
- JVA-4316 is a promising new lead peptide that significantly reduced stress-induced reinstatement of cocaine CPP in mice after pretreatment with 10 mg/kg, p.o., demonstrating comparable potency to the small molecule KOR antagonist navacaprant that was in clinical trials for major depression

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