

Development of Centrally Acting Peptide Analogs: Structure-Transport Studies and Pharmacological Evaluation of Analogs of the Opioid Peptide Dynorphin A

Jane V. Aldrich,^{1*} Kshitij A. Patkar,¹ Arvind K. Chappa,² Weijie Fang,¹ Kenneth L. Audus,² Susan M. Lunte,² Amanda N. Carey³ and Jay P. McLaughlin³

¹Department of Medicinal Chemistry and ²Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.; ³Department of Psychology, Northeastern University, Boston, MA 02155, U.S.A.

E-mail: jaldrich@ku.edu

Introduction

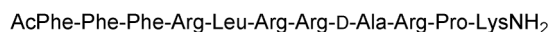
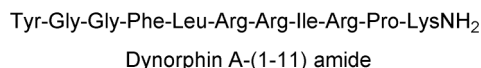
Opioid receptors are major targets for the management of pain. Opiates such as morphine that exert their effects primarily through activating mu opioid receptors (MOR) are potent analgesics, but these agents are associated with serious clinical liabilities (e.g. respiratory depression and dependence), prompting the search for opioid receptor ligands devoid of these unwanted side effects [1].¹

There is considerable interest in developing ligands for kappa opioid receptors (KOR), not just as potential analgesics, but also for a variety of other therapeutic applications. KOR agonists can produce analgesia without the liabilities associated with MOR agonists [2]. They also exhibit anti-inflammatory activity [3] and neuroprotective effects [4],⁴ suppress HIV-1 expression [5,6], and acutely suppress the rewarding effects of cocaine [7]. While KOR antagonists have been extensively used as pharmacological tools, they also show promise as therapeutic agents, with recent reports describing antidepressant effects [8], activity against opiate dependence [9] and the ability to prevent stress-induced reinstatement of cocaine-seeking behavior [10,11]. The nonpeptide KOR-selective antagonists, however, have consistently shown exceptionally long activity, lasting weeks to more than a month after a single dose [12,14], an effect that could greatly limit their therapeutic use.

We are interested in exploring peptide ligands for KOR *in vivo*, particularly KOR antagonists, with the goal of developing potential therapeutic agents. Peptide KOR antagonists are expected to exhibit a shorter duration of action than the nonpeptide KOR antagonists, thereby avoiding the prolonged activity associated with the nonpeptide ligands. In order for the peptide KOR ligands to be active centrally following systemic administration, they must be stable to metabolic degradation and cross the blood-brain barrier (BBB). Earlier studies demonstrated that E2078 ([NMeTyr¹,NMeArg⁷,D-Leu⁸]dynorphin A-(1-8) N-ethyl amide), an analog of the endogenous opioid peptide dynorphin A (Dyn A) stabilized to metabolic degradation, can cross the BBB [15] and produces analgesia in humans following intramuscular injection comparable to the nonpeptide KOR analgesic pentazocine [16]. These results demonstrate the therapeutic potential of peptide ligands for KOR.

Therefore we examined several Dyn A-(1-11) amide analogs for their ability to cross the BBB and metabolic stability. Our *in vivo* studies focused on two selective KOR peptide antagonists developed in our laboratory,

arodyn (Ac[Phe^{1,2,3},Arg⁴,D-Ala⁸]Dyn A-(1-11) amide [17]) and [N-benzyITyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide [18] (Figure 1), as they exhibit high selectivity for KOR (K_i ratios (KOR/MOR/DOR (delta opioid receptor)) = 1/174/583 and 1/194/>330, respectively) and nanomolar antagonist potencies against KOR *in vitro*. The results of these initial studies are described below.



Arodyn

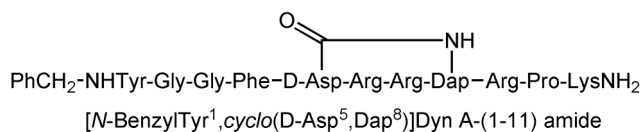


Fig. 1. Structures of Dyn A-(1-11) amide, arodyn and [N-benzyITyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide.

Results and Discussion

Several Dyn A-(1-11) amide analogs were examined for their ability to cross the bovine brain microvessel endothelial cell (BBMEC) model of the BBB [19] and for their metabolic stability. Tandem mass spectrometry following HPLC separation was used to quantify the peptides. While all of the peptides crossed the BBMEC model at least as well as the parent peptide Dyn A-(1-11) amide (Table 1), the cyclic analog *cyclo*[D-Asp⁵,Dap⁸]Dyn A-(1-11) amide exhibited 3-fold higher transport across the BBMEC than the parent linear peptide. Thus these peptides can cross the BBB to reach KOR in the central nervous system (CNS).

The metabolic stability of the peptides is dependent on the specific modifications to the peptides. Peptides with both N- and C-terminal modifications exhibit half lives in rat plasma of 1.5-2 hours (Table 2). As expected, peptides without N-terminal modification were rapidly metabolized (Table 2), presumably by aminopeptidases. Similar results have been reported for Dyn A-(1-13) analogs modified at the N- and/or C-terminus [20]. N- and C-terminal modifications, however, were not sufficient to prevent metabolism of the peptides in rat brain homogenate, and all of the peptides were rapidly degraded in this system ($t_{1/2} \leq 10-11$ minutes). The rupture of cell

membranes by homogenization would release intracellular proteases that could metabolize these peptides, however, so this system may not accurately reflect the metabolism of the peptides that would occur *in vivo*. Endopeptidases capable of metabolizing Dyn A are also present on red blood cells [21], and can rapidly metabolize some Dyn A-(1-11) amide analogs, e.g. arodyn.

Table 1. BBMEC permeability of selected dynorphin A-(1-11) amide analogs

Dyn A-(1-11) amide analog	Permeability (cm/sec) x 10 ⁶	Relative to Dyn A	Efflux ratio ^a
Arodyn	7.6 ± 3.0	2.0	1.6
cyclo[D-Asp ⁵ ,Dap ⁸]	12.2 ± 2.7	3.2	1.1
[N-BenzylTyr ¹]	4.1 ± 0.6	1.1	2.6
Ac-Dyn A-(1-11)NH ₂	7.1 ± 2.3	1.9	2.4
Dyn A-(1-11)NH ₂	3.8 ± 1.3	1.0	0.9

^a Permeability (basal to apical)/permeability (apical to basal)

Table 2. Metabolic stability of selected dynorphin A-(1-11) amide analogs

Dyn A-(1-11) amide analog	Rat plasma t _{1/2} (min)
Arodyn	104 ± 4
cyclo[D-Asp ⁵ ,Dap ⁸]	< 2 ^a
[N-BenzylTyr ¹]	90 ± 19
Ac-Dyn A-(1-11)NH ₂	111 ± 17
Dyn A-(1-11)NH ₂	< 2 ^a

^a Substrates for aminopeptidases

Selected Dyn A-(1-11) amide analogs were evaluated *in vivo* using C57Bl/6 mice for their ability to antagonize the analgesic activity of the nonpeptide KOR agonist U50,488 in the 55°C warm-water tail-withdrawal assay [22]. Following intracerebroventricular (i.c.v.) administration arodyn (0.3 nmol) significantly antagonizes U50,488 antinociception for up to 3 days [11], which is substantially shorter than the long lasting antagonism exhibited by the nonpeptide KOR selective antagonists nor-BNI and JDTC which lasts for at least 7 days [12-14].

Because arodyn is subject to rapid metabolism by endopeptidases in rat brain homogenate and whole blood (data not shown), we examined [N-benzylTyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide (JVA-1415) *in vivo*. Preliminary metabolism studies show that this cyclic peptide exhibits substantially greater metabolic stability than the linear peptide arodyn in rat brain homogenate, with a half life of 70 minutes, and is stable in whole blood for at least three hours. JVA-1415 antagonizes the analgesic effects of U50,488 (administered intraperitoneally, i.p.) following both i.c.v. (data not shown) and subcutaneous (s.c.) administration (Figure 2) in a dose dependent manner. To verify that peripherally administered peptide antagonizes U50,488 at KOR in the CNS, U50,488 was administered i.c.v. (40 nmol per mouse) 60 min after s.c. (3 mg/kg) pretreatment with JVA-1415 (Figure 3). The antagonism of analgesia mediated by centrally administered U50,488 by JVA-1415

given peripherally is strong evidence that the peptide crosses the BBB to act on KOR in the CNS.

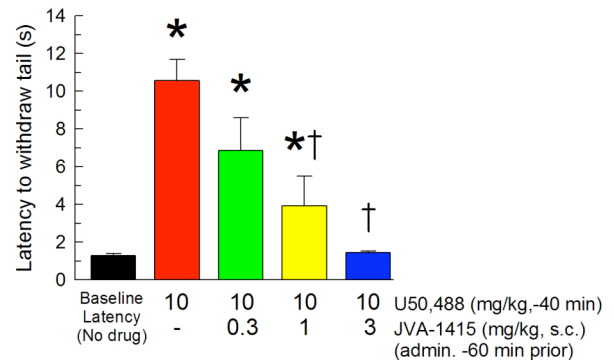


Fig. 2. *In vivo* antagonism of U50,488 by [N-benzylTyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide (JVA-1415) following s.c. administration in the 55 °C warm water tail withdrawal assay. * = significantly different ($p < 0.05$) from baseline, † = significantly different ($p < 0.05$) from U50,488 alone.

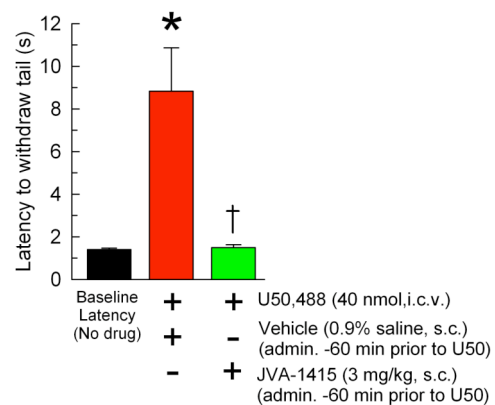


Fig. 3. Centrally mediated analgesia induced by U50,488 is antagonized by [N-benzylTyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide (JVA-1415) following s.c. administration in the 55 °C warm water tail withdrawal assay. * = significantly different ($p < 0.05$) from baseline, † = significantly different ($p < 0.05$) from U50,488 alone.

In conclusion, dynorphin A analogs containing 11 amino acids with molecular weights of approximately 1500 can cross the BBB to reach KOR in the CNS. While N- and C-terminal modified analogs exhibit enhanced metabolic stability, they are still prone to metabolism by endopeptidases. The cyclic Dyn A analog [N-benzylTyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide exhibits enhanced metabolic stability compared to the linear peptides. This peptide antagonizes central KOR *in vivo* following peripheral administration (s.c.). Peptide KOR ligands have potential as therapeutic agents, with [N-benzylTyr¹,cyclo(D-Asp⁵,Dap⁸)]Dyn A-(1-11) amide representing an important lead peptide for further development. These studies are currently underway in our laboratories.

Acknowledgments

This research was supported by grants from the National Institute on Drug Abuse (R01 DA018832 and R01 DA023924) and a General Research Fund grant from the University of Kansas.

References

1. Aldrich, J. V., and Vigil-Cruz, S. C. In *Burger's Medicinal Chemistry & Drug Discovery*, 6th ed.; Abraham, D. J., Ed. John Wiley & Sons, Inc.: New York, 2003; Vol. 6, pp 329-481.
2. Millan, M. J. (1990) *Trends Pharmacol. Sci.*, **11**, 70-76.
3. Binder, W., Carmody, J., and Walker, J. (2000) *J. Pharmacol. Exp. Ther.*, **292**, 303-309.
4. Tortella, F. C., and Decoster, M. A. (1994) *Clin. Neuropharmacol.*, **17**, 403-416.
5. Chao, C. C., Gekker, G., Hu, S., Sheng, W. S., Shark, K. B., Bu, D.-F., Archer, S., Bidlack, J. M., and Peterson, P. K. (1996) *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 8051-8056.
6. Peterson, P. K., Gekker, G., Lokensgard, J. R., Bidlack, J. M., Chang, A. C., Fang, X., and Portoghese, P. S. (2001) *Biochem. Pharmacol.*, **61**, 1145-1151.
7. Mello, N., and Negus, S. S. (2000) *Ann. N. Y. Acad. Sci.*, **909**, 104-132.
8. Mague, S. D., Pliakas, A. M., Todtenkopf, M. S., Tomasiewicz, H. C., Zhang, Y., Stevens, W. C. J., Jones, R. M., Portoghese, P. S., and Carlezon, W. A. J. (2003) *J. Pharmacol. Exp. Ther.*, **305**, 323-330.
9. Rothman, R. B., Gorelick, D. A., Heishman, S. J., Eichmiller, P. R., Hill, B. H., Norbeck, J., and Liberto, J. G. (2000) *J. Substance Abuse Treat.*, **18**, 277-281.
10. Beardsley, P. M., Howard, J. L., Shelton, K. L., and Carroll, F. I. (2005) *Psychopharmacology (Berl)*, **183**, 118-126.
11. Carey, A. N., Borozny, K., Aldrich, J. V., and McLaughlin, J. P. (2007) *Eur. J. Pharmacol.*, **569**, 84-89.
12. Horan, P., Taylor, J., Yamamura, H. I., and Porreca, F. (1992) *J. Pharmacol. Exp. Ther.*, **260**, 1237-1243.
13. Carroll, I., Thomas, J. B., Dykstra, L. A., Granger, A. L., Allen, R. M., Howard, J. L., Pollard, G. T., Aceto, M. D., and Harris, L. S. (2004) *Eur. J. Pharmacol.*, **501**, 111-119.
14. Metcalf, M. D., and Coop, A. (2005) *Aaps J*, **7**, E704-722.
15. Terasaki, T., Deguchi, Y., Sato, H., Hirai, K., and Tsuji, A. (1991) *Pharmaceut Res*, **8**, 815-820.
16. Fujimoto, K., and Momose, T. (1995) *Jap. J. Anesth*, **44**, 1233-1237.
17. Bennett, M. A., Murray, T. F., and Aldrich, J. V. (2002) *J. Med. Chem.*, **45**, 5617-5619.
18. Patkar, K. A., Yan, X., Murray, T. F., and Aldrich, J. V. (2005) *J. Med. Chem.*, **48**, 4500-4503.
19. Audus, K. L., and Borchardt, R. T. (1986) *Pharm. Res.*, **3**, 81-87.
20. Al-Fayoumi, S. I., Brugos, B., Arya, V., Mulder, E., Eppler, B., Mauderli, A. P., and Hochhaus, G. (2004) *Pharm. Res.*, **21**, 1450-1456.
21. Dando, P. M., Brown, M. A., and Barrett, A. J. (1993) *Biochem. J.*, **294 (Pt 2)**, 451-457.
22. McLaughlin, J. P., Hill, K. P., Jiang, Q., Sebastian, A., Archer, S., and Bidlack, J. M. (1999) *J. Pharmacol. Exp. Ther.*, **289**, 304-311.