Receptors & Clinical Investigation 2015; 2: e517. doi: 10.14800/rci.517; © 2015 by Yeon Sun Lee, *et al*.
http://www.smartscitech.com/index.php/rci

RESEARCH HIGHLIGHT

Blockade of non-opioid excitatory effects of spinal dynorphin A at bradykinin receptors

Yeon Sun Lee[1], Sara M. Hall[1], Cyf Ramos-Colon[1], Michael Remesic[1], David Rankin[2], Todd W. Vanderah[2], Frank Porreca[2], Josephine Lai[2], Victor J. Hruby[1]

[1]*Department of Chemistry and Biochemistry, The University of Arizona, Tucson, AZ 85721, USA*
[2]*Department of Pharmacology, The University of Arizona, Tucson, AZ 85721, USA*

Correspondence: Yeon Sun Lee and Victor J. Hruby
E-mail: yeon@email.arizona.edu (Y.S. Lee) and hruby@email.arizona.edu (V.J. Hruby)
Received: January 08, 2015
Published online: March 11, 2015

Dynorphin A (Dyn A) is an endogenous opioid ligand that possesses neuroinhibitory (antinociceptive) effects via μ, δ, and κ opioid receptors. However, under chronic pain conditions, up-regulated spinal Dyn A can also interact with bradykinin receptors (BRs) to promote hyperalgesia through a neuroexcitatory(pronociceptive) effect. These excitatory effects cannot be blocked by an opioid antagonist, and thus are non-opioid in nature. On the basis of the structural dissimilarity between Dyn A and endogenous BR ligands, bradykinin(BK) and kallidin (KD), Dyn A's interaction with BRs could not be predicted, and provided an opportunity to identify a novel potential neuroexcitatory target. Systematic structure-activity relationship (SAR) studies discovered a minimum pharmacophore of Dyn A, [des-Arg^7]-Dyn A-(4-11) LYS1044 for antagonist activity at the BRs, along with insights into the key structural features for BRs recognition, i.e., amphipathicity. The des-Tyr fragment of dynorphin does not bind to opioid receptors. Intrathecal administration of des-Tyr dynorphin produces hyperalgesia reminiscent of behaviors seen in peripheral neuropathic pain models and at higher doses, neurotoxicity. Our lead ligand LYS1044 negatively modulated Dyn A-(2-13)-induced neuroexcitatory effects in naïve animals and blocked mechanical hypersensitivity and thermal hyperalgesia in a dose-dependent manner in animals with experimental neuropathic pain. Based on these results, ligand LYS1044 might prevent abnormal pain states by blocking the neuroexcitatory effects of increased levels of Dyn A that are seen in experimental models of neuropathic pain and that likely promote excitation mediated by BRs in the spinal cord.

To cite this article: Yeon Sun Lee, *et al*. Blockade of non-opioid excitatory effects of spinal dynorphin A at bradykinin receptors. Receptor Clin Invest 2015; 2: e517. doi: 10.14800/rci.517.

Neuropathic pain is a chronic disease that is caused by injury to peripheral or central nerves. Neuropathic pain is characterized by ongoing pain and in many patients, hyperalgesia (enhanced pain to a noxious stimulus) and allodynia (i.e., pain to normally non-painful stimuli). The treatment of chronic neuropathic pain using opioids is very limited due to serious side effects such as tolerance, addiction, and constipation upon long term administration [1-2]. Current treatment of modalities for chronic neuropathic pain, especially opioids, can cause hyperalgesia with prolonged use [3]. This has been linked to changes in gene expression that are related to treatment attempts reflecting adaptive plasticity of the nervous system [4]. Therefore, to treat chronic neuropathic pain efficiently without serious side effects, there is a need to develop drugs through novel approaches considering the possible changes in pain pathways.

From this point of view, dynorphin A (Dyn A, H-Tyr^1-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln^{17}-OH), which is one of three endogenous opioid ligands along with enkephalin and endorphin, and a

major proteolytic fragment of prodynorphin that exhibits antinociceptive actions via μ, δ, and κ opioid receptors, might be a good target to pursue with regard to possible system changes. The physiological role of Dyn A is somewhat more obscure than the two endogenous ligands, enkephalin and endorphin, that are well known to elicit inhibitory effects, predominantly on neuronal cells, to elicit analgesia. Dyn A possesses very distinctive biological roles in the pain pathway, including both neuroinhibitory and neuroexcitatory effects. While Dyn A's neuroinhibitory effects are well understood along with its opioid receptor interactions, the mechanism of its neuroexcitatory effects are not yet established.

Many experimental models of pathological pain such as neuropathic and inflammatory pain and hyperalgesia show elevated levels of Dyn A in the spinal cord [5-8]. Spinal administration of an anti-Dyn A antiserum blocks pain caused by peripheral nerve injury [7, 9] and opioid-induced hyperalgesia [8], but does not change standard sensory thresholds in non-injured animals. This suggests that to maintain chronic pain states in these models, the elevated level of spinal Dyn A is necessary [10]. In prodynorphin gene mutated transgenic mice, nerve injury causes abnormal pain, but such pain does not persist. This indicates a requirement for elevation of spinal Dyn A for persistent neuropathic pain [9]. These observations suggest that approaches to block the excitatory effects of Dyn A in the central nervous system (CNS) have a high potential for therapeutic relevance. Here, we highlight Dyn A as a ligand for bradykinin receptors (BRs) in the CNS.

Invivo, Dyn A is quickly degraded to [des-Tyr^1]-Dyn A fragments by aminopeptidases in the synapse [11], resulting in the inactivation of Dyn A's inhibitory opioid actions because [des-Tyr^1]-Dyn A fragments do not interact with opioid receptors [12]. Instead, Dyn A and its [des-Tyr^1]-Dyn A fragments (e.g. Dyn A-(2-13)) produce pronociceptive and excitotoxic effects such as tactile hypersensitivity, thermal hyperalgesia,and paralysis, which cannot be blocked by naloxone, an opioid antagonist [13-16]. It is clear that Dyn A is different from the other prodynorphin-derived endogenous ligands, namely Dyn B and neo-endorphin, by its neuronal excitotoxicity and excitatory actions, which are non-opioid in nature [17].

Our studies showed that Dyn A and Dyn A-(2-13) bring about an increase in Ca^{2+} influx through voltage dependent Ca^{2+} channels by interaction with BRs in a dorsal root ganglion X neuroblastoma hybrid cell line, F-11 [10]. Considering the lack of structural similarity between Dyn A and the endogenous ligands for the BRs, bradykinin (BK) and kallidin (KD), Dyn A's interaction with the BRs could not be predicted, and provided an opportunity to identify a putative direct neuroexcitatory target. Dyn A competed with the binding of [^{3}H]BK and [^{3}H][des-Arg^{10}, Leu^9]-KD (DALKD) in brain tissues as well as cell lines that express bradykinin 2 receptors (B2Rs) [10]. Interestingly, in our recent studies, BK, DALKD, and HOE140, a well-known B2R antagonist, showed distinct binding profiles for central BRs from that previously reported in other tissues [18]. The binding affinity of BK in rat brain membranes using [^{3}H]BK or [^{3}H]DALKD is less than that shown previously for the B2R(nanomolar range), which is the major BR subtype constitutively expressed in all tissues [19-22]. Alternatively, DALKD, a bradykinin type 1 receptor (B1R) selective antagonist, interacts with the BRs in rat brain membranes with the same range of affinity as BK [20,23]. HOE140, a B2R selective antagonist, showed very low binding affinity against [^{3}H]BK in rat brain membranes. These data from rat brain BR binding sites contrast significantly to that using guinea pig ileum (GPI) where both BK and HOE140 showed high binding affinity similar to that previously reported [19-21]. Therefore, in the rat central nervous system, we may be targeting a pharmacologically distinct neuronal BR than what has been previously defined in tissues.

On the basis of Dyn A's neuronal excitatory effects in the CNS, we hypothesized that Dyn A structure-based BR antagonists could be discovered to modulate hyperalgesia in chronic neuropathic pain states. The main strategy for the rational design of BR antagonists is to identify the key structural feature, i.e. pharmacophore, of Dyn A that interacts directly with the BRs as an agonist, and then proceed to modify the structure for the binding site by scrutinizing the effects of different substituents which will lead to an antagonist [24]. Therefore, systematic structure-activity relationship (SAR) studies at rat brain BRs were performed, and as a result, a good pharmacophore, [des-Arg^7]-Dyn A-(4-11) **LYS1044**, was identified along with a key structural feature for the receptors, namely amphipathicity [18, 25-27]. It was shown that the deletion of Arg^7 residue in Dyn A analogues does not affect binding affinities while maintaining amphipathicity [26]. This SAR result is remarkable because two Arg residues in positions 6 and 7 are known to play an important role in the biological activity of Dyn A and in general, removal of one amino acid residue in the middle of a bioactive sequence typically causes significant changes of topographical structure and biological profile [23, 28]. In contrast, our SAR results confirmed that all of the [des-Arg^7]-Dyn A analogues show the same range of binding affinities to the BRs as respective Dyn A analogues. It was also shown that the BRs recognition of the Dyn A analogues is predominantly dependent upon the basicity of the C-terminal amino acid residue and is pH sensitive. Modification of the C-terminal carboxylate group to an

amide reduced the binding affinity dramatically and lowering pH 7.4, the physiological condition in medicine and biology, to 6.8 enhanced the binding affinity by 2-8 folds. The enhancement of binding affinities at a lower pH along with a vital role of basic amino acid residue at the C-terminus suggests that Dyn A recognize the BRs mainly through electrostatic interactions with the receptors and thus to improve their interactions, allocation of positive charges in ligands may be critical. Further modifications of the amphipathic ligand **LYS1044** by replacing $Arg^{6, 9}$ (and/or Lys^{11}) and Leu^{5} (and/or Ile^{8}) with other basic and hydrophobic amino acid residues, respectively, retained the same binding affinities for BRs. Therefore, we conclude that amphipathicity is key structural feature for rat brain BR recognition.

As mentioned earlier, our SAR study discovered **LYS1044**, which showed the same high binding affinity for rat brain BRs as Dyn A, to be a good pharmacophore for the receptors. Intrathecal (i.th.) administration of Dyn A (2-13) decreased paw withdrawal latency and threshold in radiant heat test (thermal hyperalgesia) and von Frey test (mechanical hypersensitivity), respectively, using naïve rats. In the same model, co-administration of **LYS1044** and Dyn A-(2-13) blocked the hyperalgesia and paralysis induced by Dyn A-(2-13) when given alone. These results suggest that ligand **LYS1044** can effectively block abnormal pain states that are induced by Dyn A-(2-13) and presumably mediated by spinal BRs, as an antagonist. In a model of peripheral neuropathy, unilateral L_5/L_6 spinal nerve ligation (SNL) injury, i.th. administration of ligand **LYS1044** blocked mechanical hypersensitivity and thermal hyperalgesia in a dose-dependent manner. Ligand **LYS1044** also inhibited the response of wide dynamic range (WDR) neurons to innocuous and noxious mechanical stimuli in neuropathic, but not naïve, animals, while Dyn A-(2-13) facilitates the response [29]. In naïve animals, ligand **LYS1044** decreased the WDR neuronal response induced by Dyn A-(2-13). It is clear that by mimicking pro-excitatory pharmacological changes with pharmacological Dyn A-(2-13), we induced a state whereby the inhibitory effects are now valid.

The inhibitory actions of **LYS1044** may be localized in the CNS since there is no peripheral activity observed in vivo. Local, intraplantar (i.pl.), administration of **LYS1044** did not show any effect on BK-induced paw edema and plasma extravasation. In contrast, co-administration of HOE140, a BK-based B2R antagonist, reduced the BK-induced paw volume increase. This result suggests that **LYS1044** does not block BK-action at the peripheral BRs, and there will be little effect on the BK's cardiovascular function at the region. At high doses, Dyn A-(2-13) showed severe motor deficiency in vivo. In contrast, **LYS1044** did not show any toxic effect or motor deficiency, and furthermore, blocked Dyn A-(2-13)-induced paralysis in vivo. The ligand represents a simple amphipathic structure and nonetheless did not bind to the other 43 off-target receptors including the three opioid receptors [30]. This result supports that the ligand's interaction with BRs is specific and our strategies successfully distinguished non-opioid activities from opioid activities.

Together, with our previous studies characterizing the role of spinal BRs to promote pain, the ability of **LYS1044** to reverse abnormal pain states by blocking the neuroexcitatory effects of increased levels of Dyn A underscores the potential role of spinal BRs as a therapeutic target for neuropathic pain. CNS distribution of BRs is well documented, but endogenous ligands of central BRs are unknown. Therefore, our experimental data may reveal an endogenous ligand for central BRs and a novel target for endogenous Dyn A neuropeptide.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgements

This work has been supported by U.S. Public Health Services, NIH, and NIDA P01DA006284 and R01DA013449. Off-target screening was provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-025C directed by Bryan L. Roth at the University of North Carolina at Chapel Hill.

References

1. King T, Ossipov MH, Vanderah TW, Porreca F, Lai J. Is paradoxical pain induced by sustained opioid exposure an underlying mechanism of opioid antinociceptive tolerance? Neurosignals 2005; 14: 194-205.
2. Foley KM. Misconceptions and controversies regarding the use of opioids in cancer pain. Anticancer Drugs 1995; 6: 4-13.
3. Vanderah TW, Ossipov MH, Lai K, Malan TP Jr, Porreca F. Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. Pain 2001; 92: 5-9.
4. Hruby VJ, Porreca F, Yamamura HI, Tollin G, Agnes R, Lee YS, *et al*. New paradigms and tools in drug design for pain and addiction. AAPS J 2006; 8: E450-E460.
5. Ruda MA, Iadarola MJ, Cohen LV, Young WS. In situ hybridization histochemistry and immunocytochemistry reveal an increase in spinal dynorphin biosynthesis in a rat model of peripheral inflammation and hyperalgesia. Proc Natl Acad Sci USA 1988; 85: 622-626.

6. Draisci G, Kajander KC, Dubner R, Bennett GJ, Iadarola MJ. Up-regulation of opioid gene expression in spinal cord evoked by experimental nerve injuries and inflammation. Brain Res 1991; 560: 186-192.

7. Malan TP Jr, Ossipov MH, Ibrahim M, Bian DI, Lai J, Porreca F. Extraterritorial neuropathic pain correlates with multi-segmental elevation of spinal dynorphin in nerve-injured rats. Pain 2000; 86: 185-194.

8. Vanderah TW, Gardell LR, Burgess SE, Ibrahim M, Dogrul A, Zhong C, *et al*. Dynorphin promotes abnormal pain and spinal opioid antinociceptive tolerance. J Neurosci 2000; 20: 7074-7079.

9. Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U, *et al*. Pronociceptive actions of dynorphin maintain chronic neuropathic pain. J Neurosci 2001; 21: 1779-1786.

10. Lai J, Luo M, Chen Q, Ma S, Gardell LR, Ossipov MH, et al. Dynorphin A activates bradykinin receptors to maintain neuropathic pain. Nat Neurosci 2006; 9: 1534-1540.

11. Young EA, Walker JM, Houghten R, Akil H. The degradation of dynorphin A in brain tissue in vivo and in vitro. Peptides 1987; 8: 707-707.

12. Walker JM, Moises HC, Coy DH, Baldrighi G, Akil H. Nonopiate effects of dynorphin and des-Tyr-dynorphin. Science 1982; 218: 1136-1138.

13. Vanderah TW, Laughlin T, Lashbrook JM, Nichols ML, Wilcox GL, Ossipov MH, *et al*. Single intrathecal injection of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats; blockade by MK-801 but not naloxone. Pain 1996; 68: 275-281.

14. Faden AI, Jacobs TP. Dynorphin-related peptides cause motor dysfunction in the rat through a non-opiate action. Br J Pharmacol 1984; 81: 271-276.

15. Faden AI. Dynorphin increases extracellular levels of excitatory amino acids in the brain through a non-opioid mechanism. J Neurosci 1992; 12: 425-429.

16. Long JB, Petras JM, Mobley WC, Holaday JW. Neurological dysfunction after intrathecal injection of dynorphin A (1-13) in the rat. II. Nonopioid mechanisms mediate loss of motor, sensory and autonomic function. J Pharmacol Exp Ther 1988; 146: 1157-1174.

17. Tan-no K, Esashi A, Nakagawasai O, Niijima F, Tadano T, Sakurada C, *et al*. Intrathecally administered big dynorphin, a prodynorphin-derived peptide, produces nociceptive behavior through an *N*-methyl-D-aspartate receptor mechanism. Brain Res 2002; 952: 7-14.

18. Lee YS, Muthu D, Hall SM, Ramos-Colon C, Rankin D, Hu J, *et al*. Discovery of amphipathic dynorphin A analogues to inhibit the neuroexcitatory effects of dynorphin A through bradykinin receptors in the spinal cord. J Am Chem Soc 2014; 136: 6608-6616.

19. Marceau F, Hess JF. Bachvarov DR.The B1 receptors for kinins. Pharm Rev 1998; 50: 357-386.

20. Innis RB, Manning DC, Stewart JM, Snyder SH. [^{3}H]Bradykinin receptor binding in mammalian tissue membranes. Proc Natl Acad Sci USA 1981; 78: 2630-2634.

21. Liebmann C, Bosse R, Escher E. Discrimination between putative bradykinin B2 receptor subtypes in guinea pig ileum smooth muscle membranes with a selective iodinatable bradykinin analogue. Mol Pharm 1994; 46: 949-956.

22. Hess JF, Borkowski JA, Macneil T, Stonesifer GY, Fraher J, Strader CD, *et al*. Differential pharmacology of cloned human and mouse B2 bradykinin receptors. Mol Pharm 1993; 45: 1-8.

23. Chavkin C, Goldstein A. Specific receptor for the opioid peptide dynorphin; structure-activity relationships. Proc Natl Acad Sci 1981; 78: 6543-6547.

24. Hruby VJ. Designing peptide receptor agonists and antagonists. Nat Rev Drug Discovery 2002; 1: 847-858.

25. Lee YS, Hruby VJ, Lai J, Porreca F. Discovery of Novel Amphipathic Ligands for the Dynorphin Binding Site on the Bradykinin Receptor. PCT Int. Appl. 2014; WO 2014190313.

26. Lee YS, Rankin D, Hall SM, Ramos-Colon C, Ortiz JJ, Kupp R, *et al*. Structure-activity relationships of non-opioid [des-Arg^7]-dynorphin A analogues for bradykinin receptors. Bioorg Med Chem Letts 2014; 24: 4976-4979.

27. Lee YS, Hall SM, Ramos-Colon C, Remesic M, LeBaron L, Nguyen A, *et al*. Modification of amphipathic non-opioid dynorphin A analogues for rat brain bradykinin receptors. Bioorg Med Chem Letts 2015; 25: 30-33.

28. Turcotte A, Lalonde JM, St-Pierre S, Lemaire S. Dynorphin-(1-13). I. Structure-function relationships of Ala-containing analogs. Int J Pept Protein Res 1984; 23: 361-367.

29. Bannister K, Lee YS, Goncalves L, Porreca F, Lai J, Dickenson AH. Neuropathic plasticity in the opioid and non-opioid actions of dynorphin A fragments and their interactions with bradykinin B2 receptors on neuronal activity in the rat spinal cord. Neuropharmacology 2014; 85: 375-383.