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

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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-Arg²]-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-Arg 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.


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-Tyr¹-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln¹⁷-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 \( \mu \), \( \delta \), and \( \kappa \) 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 antisera 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-Tyr1]-Dyn A fragments by aminopeptidases in the synapse [11], resulting in the inactivation of Dyn A’s inhibitory opioid actions because [des-Tyr1]-Dyn A fragments do not interact with opioid receptors [12]. Instead, Dyn A and its [des-Tyr1]-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 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 \( \text{Ca}^{2+} \) influx through voltage dependent \( \text{Ca}^{2+} \) 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 \([\text{H}]\text{BK}\) and \([\text{H}][\text{des-Arg}^{10}, \text{Leu}^2]\)-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 \([\text{H}]\text{BK}\) or \([\text{H}]\text{DALKD}\) 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 \([\text{H}]\text{BK}\) 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-Arg7]-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 Arg7 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-Arg7]-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 Arg⁶,⁹ (and/or Lys⁴) and Leu⁵ (and/or Ile⁸) 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 L⁶/L⁶ 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. 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. This result suggests 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.

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