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Amphipathic Non-opioid Dynorphin A Analogs to Inhibit Neuroexcitatory Effects at Central Bradykinin Receptors

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Introduction

Nerve injury and inflammation cause up-regulation of 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) in the spinal cord, which results in hyperalgesia *via* the interaction with bradykinin receptors (BRs) [1]. This is a non-opioid neuroexcitatory effect that cannot be blocked by an opioid antagonist, naloxone. On the basis of the fact, systematic structure-activity relationship study on Dyn A was performed to develop BRs antagonists that can block the hyperalgesia. As a result, **LYS1044**, [des-Arg[7]]-Dyn A-(4-11), was identified as a lead ligand along with key insights into structural features for the BRs recognition (i.e. amphipathicity) [2-4]. Intrathecal administration of the lead ligand reversed thermal hyperalgesia and mechanical hypersensitivity in nerve injured animals and inhibited non-opioid Dyn A-induced motor impairment and hyperalgesia in naïve animals. Yet, this ligand showed very low metabolic stabilities in plasma and was completely degraded within 4 hours of incubation (half-life < 1 hour). Therefore, in an effort to improve the metabolic stability and also to enhance the blood brain barrier permeability, various modifications were performed on Dyn A structure. Here we report design and synthesis of cyclic Dyn A analogues and their biological activities.

Results and Discussion

To utilize potential advantages of cyclic peptide ligands, we first designed and synthesized two carba Dyn A analogues (**ML72-4** and **ML72-5**), which contain a 17-membered ring (Figure 1). In these structures, three Lys residues were allocated to position the side amino groups exposed but two hydrophobic amino acid residues were consumed for the cyclization, since positive charges were considered to play an important role in the BRs recognition. These two analogues lost binding affinities at the receptors (IC_{50} > 10,000 nM), which suggests that 17-membered ring may be too small or hydrophobic residues are also necessary to retain amphipathicity for the BRs. Therefore, to conserve the amphipathicity by exposing the hydrophobic alkyl chain together with positive charges, we designed cyclic Dyn A analogues (17- or 20-membered ring) in which the Pro moiety of **LYS1044** is involved in a cyclization with the *N*-terminus (Figure 2). The main consideration in our design was to enhance the turn structure around the Pro residue and to expose positive charges and hydrophobic groups properly for the ligand amphipathicity.

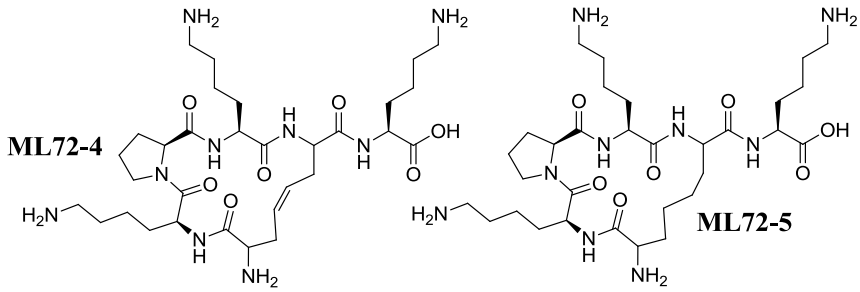

Fig. 1. Structures of Carba Dyn A Ligands.

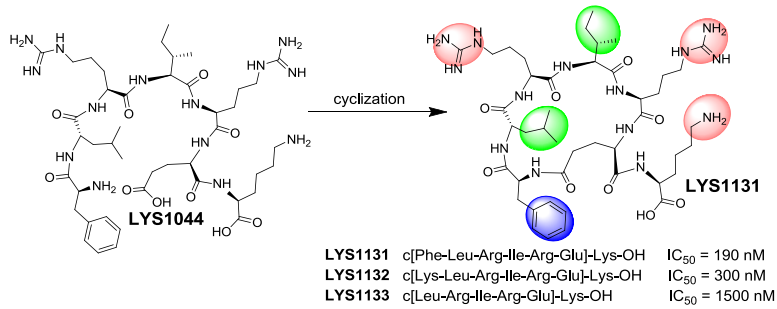

Fig. 2. Binding affinities of cyclic Dyn A analogues at BRs. Radioligand competition assays were carried out at pH 6.8 using [^{3}H]Bradykinin in rat brain membranes.

Cyclic Dyn A analogues (**LYS1131**, **LYS1132**, and **LYS1133**) were synthesized by solid phase synthesis using Fmoc-chemistry with Pip, Pbf, and Boc group as a side protecting group for Glu, Arg, and Lys, respectively, on Fmoc-Lys(Boc)-attached Wang resin. Couplings were carried out with 3 equiv HBTU / 3 equiv HOBt / 6 equiv DIPEA for 50 min and N^{α}-Fmoc groups were deprotected by 20% piperidine in DMF for 20 min. After chain elongations, Pip groups on Glu residues were deprotected by 5% TFA for 5 min 3 times and the resulting acids were consumed for a cyclization with a N^{α}-amino group using 5 equiv DIC / 5 equiv HOBt for 3 hours. The cyclic analogues were cleaved by a cocktail solution containing 90% TFA, 5% thioanisole, 3% EDT, and 2% anisole and purified by RP-HPLC to afford more than 95% pure cyclic Dyn A analogues. Binding affinities of cyclic Dyn A analogues were determined by radioligand competition analysis using [^{3}H]Bradykinin in rat brain membranes. Data was analyzed by nonlinear least-squares analysis using GraphPad Prism 4 and IC_{50} values were determined from nonlinear regression analysis of data collected from three independent experiments.

Ligand **LYS1131**, which contains a 20-membered ring, retained the same range of binding affinity (IC_{50} = 190 nM) for BRs as **LYS1044** (IC_{50} = 97 nM). Ligand **LYS1132** with the same size ring as **LYS1131** also showed the same range of affinity (IC_{50} = 300 nM) for the BRs. Even with the slight loss of affinities, it is clear that the cyclization between Pro residue position and the *N*-terminus is well tolerated for the receptor. Ligand **LYS1133** which contains a 17-membered ring, decreased affinity (IC_{50} = 1500 nM), but showed better affinity than two carba cyclic analogues **ML72-4** and **ML72-5**. This SAR results indicate that hydrophobic residues play a role in the BRs recognition, too. Taken together, it is suggested that 20-membered ring and amphipathicity are key structural features of cyclic Dyn A analogues for the BRs recognition.

Acknowledgments

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