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Modification of amphipathic non-opioid dynorphin A analogues for rat brain bradykinin receptors

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ABSTRACT

It has been shown that under chronic pain or nerve injury conditions, up-regulated dynorphin A (Dyn A) interacts with bradykinin receptors (BRs) to cause hyperalgesia in the spinal cord. Thus BRs antagonist can modulate hyperalgesia by blocking Dyn A's interaction with the BRs in the central nervous system. In our earlier structure–activity relationship (SAR) study, [des-Arg^7]-Dyn A-(4–11) **13** was discovered as a minimum pharmacophore for rat brain BRs with its antagonist activity (anti-hyperalgesic effect) in in vivo tests using naïve or injured animals. We have pursued further modification on the [des-Arg^7]-Dyn A analogues and identified a key insight into the pharmacophore of the rat brain BRs: amphipathicity.

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) interacts with three subtype (μ, δ, and κ) opioid receptors to inhibit nociception in the nervous system. On the other hand, Dyn A and [des-Tyr^1]-Dyn A bind to other non-opioid receptors such as bradykinin receptors (BRs) to stimulate nociception in the central nervous system (CNS), which results in motor impairment, paralysis, and hyperalgesia.[1–6] These excitatory effects are non-opioid effects, because naloxone, an opioid antagonist, cannot block these effects.

On the basis of this fact, we hypothesized that BRs antagonists can block the Dyn A's interaction with the receptors in the CNS to modulate the excitatory effects. To develop potent BRs antagonists we have been utilizing the Dyn A structure. In earlier studies, we had identified a good pharmacophore of the Dyn A for the BRs antagonism in rat brain: [des-Arg^7]-Dyn A-(4–11) (**13**).[7] Ligand **13** inhibited the hyperalgesic and motor effects that are caused by Dyn A-(2–13) in the CNS.[7,8] We also demonstrated that the deletion of Arg^7 residue in Dyn A analogues does not affect binding affinities at all while maintaining an amphipathic property.[9] Therefore, we pursued further modifications of amphipathic Dyn A analogs and gained a key insight into the pharmacophore for the receptors.

The modifications of the Dyn A analogues were performed based on the scaffold in Table 1, in which AA^1 and AA^2 are a hydrophobic amino acid (or peptide fragment) and a basic amino acid (or peptide fragment), respectively, and m is the number for the amphipathic moiety that is composed of a hydrophobic amino acid (or peptide fragment) and a basic amino acid (or peptide fragment). For the clarity, all of the Dyn A analogues were categorized by number '*m*' into five groups (*m* = 0, 1, 2, 3, and 4). Syntheses of the Dyn A analogues were performed by standard solid phase peptide synthesis using N^{α}-Fmoc-chemistry on amino acid pre-loaded Wang resin (100–200 mesh) in high yields (overall yields >40%).[7] Coupling reactions were performed using HBTU/HOBt/DIPEA (3 equiv/3 equiv/6 equiv) in DMF for 1 h at rt and N^{α}-Fmoc-group was deprotected by 20% piperidine in DMF for 20 min at rt. After completing chain-elongation, resin was dried under vacuum for 3 h and cleaved by TFA cocktail (TFA/TIS/water = 95/2.5/2.5) for 3 h at rt. In most cases, crude peptides were obtained in high purity (70–90%) and could be isolated with more than 97% purity by

Abbreviations: BK, bradykinin; BR, bradykinin receptor; CNS, central nervous system; DALKD, [des-Arg^{10},Leu^9]-kallidin; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Dyn A, dynorphin A; HR MS, high resolution mass spectroscopy; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; RP-HPLC, reverse phase high performance liquid chromatography; rt, room temperature; SAR, structure–activity relationship; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

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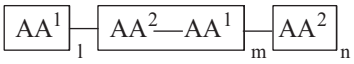
Table 1
Binding affinities of modified amphipathic non-opioid Dyn A analogs at BRs in rat brain



AA^1 = a hydrophobic amino acid (or peptide fragement);
AA^2 = a basic amino acid (or peptide fragment);
l = 0, 1; m = 0 - 4; n = 0, 1

Ligand	AA^1	*m*	$(AA^2–AA^1)_m$	AA^2	BR,[a] [^{3}H]DALKD	
					Log (IC_{50})[b]	IC_{50} (nM)
1	Gly-Phe-Leu	0		Arg	−6.02±0.08	950[c]
2	Gly-Phe-Leu		Arg-Arg-Ile		−5.63±0.27	2300[c]
3	Phe-Leu	1	Arg-Arg-Ile		−5.32±0.16	4800[c]
4	Pro		Lys-Leu	Lys	−6.68±0.10	210[d]
5	Phe		Lys-Leu	Lys	−6.46±0.13	350[c]
6	Phe(NH_2)		Lys-Leu	Lys	−6.35±0.11	450[c]
7	Pro		Lys-Nle	Lys	−7.15±0.13	71[c]
8	Nle		Lys-Nle	Lys	−6.94±0.15	110[c]
9	Ile	2	Arg-Pro-Lys-Leu	Lys	−7.31±0.09	49
10			Arg-Pro-Lys-Leu	Lys	−7.24±0.12	58
11	Nle		Lys-Pro-Lys-Nle	Lys	−7.11±0.14	78[d]
12	Nle		Arg-Pro-Arg-Nle	Lys	−7.08±0.10	83
13	Phe-Leu		Arg-Ile-Arg-Pro	Lys	−7.16±0.09	69[d]
14	Nle		Lys-Ile-Lys-Pro	Lys	−6.34±0.09	460
15	Phe-Ile		Arg-Ile-Arg-Pro	Arg	−6.96±0.11	110
16	Phe-Leu		Arg-Leu-Arg-Pro	Arg	−7.00±0.16	100
17	Phe-Nle		Arg-Nle-Arg-Pro	Arg	−7.06±0.10	87[d]
18	DPhe-DNle		DArg-DNle-DArg-DPro	DLys	n.c.	n.c.
19	Gly-Gly-Phe-Leu	3	Arg-Ile-Arg-Pro-Lys-Leu	Lys	−7.39±0.34	41[d]
20	Phe		Arg-Ile-Arg-Pro-Lys-Leu	Lys	−7.08±0.15	83
21			Arg-Ile-Arg-Pro-Lys-Leu	Lys	−7.19±0.04	65
22			Lys-Nle-Lys-Pro-Lys-Leu	Lys	−7.07±0.16	85[d]
23			Lys-Leu-Lys-Pro-Arg-Ile		−5.20±0.10	6300
24	Phe-Leu		Arg-Arg-Ile-Arg-Pro-Lys-Leu		−5.28±0.10	5200
25			Lys-Pro-Arg-Ile-Arg-Leu		−6.03±0.21	930
26			Lys-Pro-Arg-Ile-Arg-Leu	Phe[e]	−7.30±0.09	50
27			DArg-DPro-DArg-DNle-DArg-DNle	DPhe[e]	n.c.	n.c.
28	Leu-Leu		Lys-Pro-Arg-Ile-Arg-Arg-Leu	Phe-Gly-Gly[e]	−7.03±0.10	93
29		4	Lys-Leu-Lys-Pro-Arg-Ile-Arg-Leu	Phe-Gly-Gly[e]	−6.82±0.15	150
30			Lys-Leu-Lys-Pro-Arg-Ile-Arg-Arg-Leu	Phe-Gly-Gly[e]	−7.11±0.06	78

n.c. no competition.
[a] Competition assays were carried out at pH 6.8 using rat brain membranes.
[b] Logarithmic values determined from the nonlinear regression analysis of data collected from at least two independent experiments in duplicate.
[c] pH 7.4.
[d] For the comparison, IC_{50} values of respective Dyn A fragments in Reference 7 or 9 are shown in this column.
[e] The definition of AA^2 does not applied for this case.

preparative RP-HPLC using gradient (10–40% acetonitrile in water containing 0.1% TFA in 15 min) in a short time (<15 min) owing to their hydrophilic characters (Table 2). The purified Dyn A analogues were validated by analytical RP-HPLC and HR MS, and tested for their binding affinities by radioligand competition analysis using [^{3}H]DALKD in rat brain membranes where non-specific binding is defined by 10 μM kallidin.[7]

In our earlier studies, it was shown that the BRs recognition of the Dyn A analogues is predominantly dependent upon the basicity of the C-terminal amino acid residue, and that modification of the C-terminal carboxylate group to an amide reduced the binding affinity dramatically.[7] In this work, we confirmed that ligands **2**, **3**, and **23**–**25**, which do not include a C-terminal basic amino acid, bound to the BRs with very low affinity (ex. IC_{50} = 6300 nM for **23**) regardless of the length of the peptides. The vital role of a basic amino acid residue at the C-terminus for the binding again suggests that the BRs recognition is mainly through the electrostatic interactions between the receptors and ligands.

Ligand **1**, which is without an amphipathic moiety (*m* = 0) in the scaffold, showed relatively low binding affinity (IC_{50} = 950 nM) even with a basic amino acid residue at the C-terminus. This result indicated that the amphipathic moiety also plays an important role in receptor recognition along with a C-terminal basic amino acid, and thus, at least one amphipathic moiety ($m \geqslant 1$) is necessary for receptor recognition. It is worthwhile to note that still ligand **1** retained relatively higher binding affinity comparing to ligands **2** (IC_{50} = 2300 nM) and **3** (IC_{50} = 4800 nM). This comparison suggested that the most important pharmacophoric feature for the receptor recognition might be the basicity at the C-terminus.

As shown in Table 1, binding affinities of ligands (**4**–**22**) were slightly enhanced by the increase of amphipathic moiety at $1 \leq m \leq 3$. The average binding affinities of ligands with *m* = 1, 2, and 3 were 240 nM, 130 nM, and 80 nM, respectively. In fact, at *m* = 1, all ligands except **4** were tested for their binding to the BRs at higher pH (7.4) than the pH (6.8) for the other ligands (*m* = 2–4). Therefore, their binding affinities at pH 6.8 could be better than these at pH 7.4 because binding affinities of Dyn A analogues were shown to be pH sensitive and to be increased at the lower pH.[7,9] The pH condition might cause relatively higher average binding affinities compared to the others. All ligands with *m* = 2, except **14** (IC_{50} = 460 nM) showed the same range of binding affinities (average affinity: 78 nM) as ligands **19**–**22** with *m* = 3.