

A NOVEL SUPER-POTENT NEUROKININ A RECEPTOR ANTAGONIST CONTAINING DEHYDROALANINE

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Abstract – We report here the synthesis and preliminary pharmacological characterization of a novel Neurokinin A receptor antagonist. This molecule contains a dehydroalanine residue. It displays a high conformational rigidity and possesses very high activity. Its pharmacological properties as a neurokinin A receptor antagonist were assessed in *in vitro* experiments on rat vas deferens and were compared to those of Neuronorm and MEN10627. © 1998 Elsevier Science Ltd. All rights reserved.

Neurokinin A (NKA) is a member of a neuropeptide family, the tachykinins (TKs), which share a common pentapeptide sequence (Phe-Xaa-Gly-Leu-Met-NK2: Xaa: variable amino acid) and are widely distributed in the central and peripheral nervous system of mammals, including humans.¹ NKA plays an important role in a variety of physiological and pathophysiological processes, such as smooth muscle contraction in the airways, intestine and genitourinary tract,² pain processing^{3–5} and anxiety.⁶ NKA exerts these biological effects by preferentially activating the NK-2 receptor; however NKA also interacts with lower affinity with the NK-1 and NK-3 receptors, which are better activated by the other two mammalian TKs, substance P and Neurokinin B, respectively.^{7–9} Because NKA can be involved in several pathologies, NK-2 receptor antagonists are of great interest. In fact, the development of selective antagonists for the NK-2 receptor may lead to a new class of therapeutic agents for treatment of a number of human diseases.

Conformationally constrained peptides seem to be good candidates in developing receptor antagonists with enhanced potency, selectivity and enzymatic stability. The first example of a selective NK-2 receptor antagonist was the cyclic hexa-peptide L659,877, developed by McKnight *et al.*¹⁰ Structural characterization in solution by NMR spectroscopy and Molecular Dynamic calculations showed for L659,877 several conformational families to be allowed, despite its cyclic structure.^{11–14}

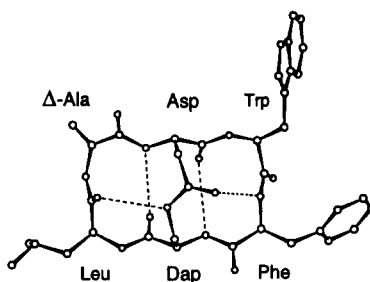
With the aim of obtaining more active and selective NK-2 receptor antagonists, we undertook the design of highly constrained hexa-peptides; the compounds that we achieved are characterized by a bicyclic structure

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obtained by a combined backbone to backbone and side chain to side chain cyclization.^{15–18} The design, synthesis, structural and pharmacological characterization of the most active compounds, namely MEN10627^{15,16} and Neuronorm,¹⁸ have recently been reported. MEN10627 is highly hydrophobic with the following sequence: cyclo[Met¹-Asp²-Trp³-Phe⁴-Dap⁵-Leu⁶]cyclo(2 β -5 β) (Dap: 2,3-diamino propionic acid). To the best of our knowledge, it is among the most potent, selective and long lasting, peptide-based NK-2 antagonists known to date.¹⁹ Neuronorm, (cyclo[Cys¹(β -D-gal)-Asp²-Trp³-Phe⁴-Dap⁵-Leu⁶]cyclo(2 β -5 β); β -D-gal = β -D-galactopyranosyl), is a water soluble analog in which the hydrophobic Met¹ residue has been replaced with the hydrophilic residue Cys¹(β -D-gal). This single amino acid substitution is sufficient to provide a water solubility of 1.8 mg/ml for Neuronorm, higher than that found for MEN10627, which is 15 μ g/ml. The NK-2 receptor antagonism of Neuronorm is comparable to that of MEN10627, as determined in *in vitro* experiments on several pharmacological preparations, not withstanding the increased hydrophilicity.²⁰

During the last step of the synthesis of Neuronorm, a treatment with methanolic sodium methoxide was performed to convert the protected cyclo[Cys¹(tetra-O-acetyl- β -D-gal)-Asp²-Trp³-Phe⁴-Dap⁵-Leu⁶]cyclo(2 β -5 β) to the unprotected Neuronorm (cyclo[Cys¹(β -D-gal)-Asp²-Trp³-Phe⁴-Dap⁵-Leu⁶]cyclo(2 β -5 β)).¹⁸ Under mild conditions (CH₃ONa concentration = 1.5 mM and reaction time > 2 h) no side-reaction was observed. In contrast, when more drastic conditions were employed (CH₃ONa concentration > 5 mM and reaction time > 5 h) the base catalyzed β -elimination occurred. In fact, a side-product was isolated by RP-HPLC and characterized by fast atom bombardment (FAB) mass spectrometry and ¹H NMR spectroscopy.²¹ It was found to correspond to cyclo[Δ -Ala¹-Asp²-Trp³-Phe⁴-Dap⁵-Leu⁶]cyclo(2 β -5 β) (Δ -Ala = dehydroalanine; see figure 1). The acronym used in the text is Δ -Ala¹-Neuronorm.

Figure 1: Schematic representation of the structure of Δ -Ala¹-Neuronorm



The activity of Δ -Ala¹-Neuronorm was assessed in *in vitro* experiments on smooth muscle preparation expressing the tachykinin NK-2 receptors,^{8,22} such as rat vas deferens (RVD). The effect of Δ -Ala¹-Neuronorm was compared to the NK-2 selective antagonists, Neuronorm and MEN10627. β -Ala⁸-NKA[4-10] was used as NK-2 agonist. Table 1 summarizes the results obtained with Δ -Ala¹-Neuronorm, Neuronorm, and MEN10627 in RVD.²³ Δ -Ala¹-Neuronorm antagonized the effect of β -Ala⁸-NKA[4-10] similarly to Neuronorm and

MEN10627. All the peptides were able to shift to the right the concentration-effect curve to β -Ala⁸-NKA[4-10]. Δ -Ala¹-Neuronorm was more potent than both Neuronorm and MEN10627. A pA_2 of 9.65 ± 0.02 was determined, while both Neuronorm and MEN10627 showed about the same pA_2 in these preparations (8.25 ± 0.04 and 8.21 ± 0.04 , respectively). The preliminary data on the pharmacological activity of Δ -Ala¹-Neuronorm, in RVD smooth muscle preparations showed that this compound is capable of competitively antagonizing NK-2 receptors. No reduction of the maximal responses to the agonist tested was observed in this preparation.

Table 1: Antagonist Activity of Δ -Ala¹-Neuronorm, Neuronorm and MEN10627 at NK-2 receptor in RVD preparations. β -Ala⁸-NKA[4-10] was used as agonist. Each value is the mean \pm S.E.M. of five preparations.²⁴

	Cont rol	β -Ala ⁸ -NKA[4-10] and Δ -Ala ¹ -Neuronorm			β -Ala ⁸ -NKA[4-10] and Neuronorm			β -Ala ⁸ -NKA[4-10] and MEN10627		
[Antag] (nM)		0.3	1	3	10	30	100	10	30	100
E _{max} (%)	100	97 \pm 2	98 \pm 1	95 \pm 3	96 \pm 1.2	99 \pm 1	92 \pm 3	95 \pm 2	96 \pm 1	94 \pm 1
pA_2		9.65 \pm 0.02			8.25 \pm 0.04			8.21 \pm 0.04		

Further investigations are in due course to test the selectivity of Neuronorm and D-Ala¹-Neuronorm toward the NK-1 and NK-3 receptors. It represents a new lead for the development of a new drug for the treatment of several diseases, including asthma. Structural analysis of D-Ala¹-Neuronorm in solution by ¹H NMR spectroscopy is presently under progress and will be published elsewhere. The structural analysis confirmed the structure for D-Ala¹-Neuronorm to be similar to that of MEN10627 (see figure 1).

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21. The $\text{CH}_3\text{ONa}/\text{CH}_3\text{OH}$ treatment used to remove the acetyl groups from the carbohydrate moiety was accidentally prolonged overnight. A 5 mM CH_3ONa solution, freshly prepared as a 0.2 M solution by dissolving Na in dry CH_3OH , was used. The purification of the reaction mixture by RP-HPLC afforded the side-product in 7 % yield, based on the initial resin substitution. FAB-Mass spectrometry gave a molecular ion peak $[\text{M}-\text{H}]^+$ of 699 amu, corresponding to $\Delta\text{-Ala}^1\text{-Neuronorm}$.
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23. The tissues were excised from albino Wistar rats (300–320 g). The experimental conditions were as previously reported.¹⁸ After the equilibrium period (90 min), tissue were electrically stimulated²² and cumulative concentration-response curves to $\beta\text{-Ala}^8\text{-NKA}[4-10]$ (0.1 to 100 nM), were obtained. Moreover, cumulative concentration-response curves for $\beta\text{-Ala}^8\text{-NKA}[4-10]$ were obtained previous incubation of $\Delta\text{-Ala}^1\text{-Neuronorm}$ (0.3 nM to 3 nM for 30 min), Neuronorm (10 nM to 100 nM for 30 min) or MEN10627 (10 nM to 100 nM for 15 min).
24. Agonist potency was expressed as EC_{50} (agonist concentration needed to reduce 50% of the maximal response) and pD_2 values (negative logarithm of EC_{50}). Schild plot analysis was performed for each antagonist in various preparations. When the results were compatible with competitive antagonism (slope of Schild not significantly different from unity), pA_2 values were calculated according to Tallarida.²⁵
All data in the text are means \pm S.E.M. of five preparations. Statistical analysis was performed by means of the Student's t test for paired and unpaired data or by means of an analysis of variance when applicable.
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