

Synthesis and biological activity of nociceptin/orphanin FQ(1–13)NH₂ analogues modified in 9 and/or 13 position

Emilia D. Naydenova,^{a,*} Vanya I. Zhivkova,^a Rositza N. Zamfirova,^b
Lubomir T. Vezekov,^a Yordanka G. Dobrinova^b and Polina I. Mateeva^b

^aDepartment of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia 1756, Bulgaria

^bInstitute of Physiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

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Abstract—The purpose of the present study was the synthesis and the biological screening of new analogues of N/OFQ(1–13)NH₂, the minimal sequence maintaining the same activity as the natural peptide nociceptin. In order to investigate the role of Lys, we substituted Lys at positions 9 and/or 13 by Orn, Dab (diaminobutanoic acid) or Dap (diaminopropanoic acid). The new N/OFQ(1–13)NH₂ analogues exerted strong and naloxone-resistant inhibition of electrically evoked contractions of rat vas deferens. Lys replacement with Orn maintained or even enhanced the inhibitory activity, while replacements with Dab and Dap decreased inhibitory activity.

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Nociceptin, also known as orphanin FQ (N/OFQ), is a neuropeptide, structurally related to opioid peptides (especially dynorphin A), which does not interact with classical opioid receptors. The heptadecapeptide N/OFQ (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) selectively activates its own receptor NOP, previously named opioid receptor-like 1 (ORL₁) or OP₄, a novel member of the opioid receptor family.^{1,2} It has been shown that the NOP-receptor and N/OFQ are expressed not only in the central nervous system (CNS) but also in peripheral tissues, suggesting that this novel peptide–receptor system might modulate both central and peripheral biological functions.^{3–6} At central sites, N/OFQ modulates pain, anxiety, feeding, locomotion, learning and memory. In periphery, it affects cardiovascular, gastrointestinal, urogenital and respiratory functions. Guerrini et al.⁷ proposed that the N-terminal tridecapeptide sequence of the nociceptin molecule suffices for its full biological activities. It has been reported that Arg,^{8,12} Lys^{9,13} seem to be crucial for receptor occupation, most probably interacting with the acidic amino acids present in the second extracellular loop of the NOP-receptor.^{8,9} Structure–activity relationship (SAR) studies demonstrated

that the N/OFQ sequence can be divided into a N-terminal tetrapeptide ‘message’ crucial for receptor activation and a C-terminal ‘address’ important for receptor binding.¹⁰

Based on the ‘message/address’ concept we aimed to investigate the importance of the Lys and distance of the side-chain amino group from the peptide backbone on the biological activity. We substituted Lys at positions 9 and/or 13 by its own structural analogues Orn, diaminobutanoic acid (Dab) or diaminopropanoic acid (Dap) in the N/OFQ(1–13)NH₂ template. Lysine was chosen on the assumption that cationic residues (as Arg,^{8,12} Lys^{9,13}) appear to play a functional role for receptor binding.^{7,10}

The solid-phase peptide synthesis by Fmoc (9-fluorenylmethoxycarbonyl) chemistry was used to obtain several structural analogues of N/OFQ(1–13)NH₂ by replacing Lys at positions 9 and/or 13 with Orn, Dab or Dap.¹¹

The crude peptides were purified on a reversed-phase high-performance liquid chromatography (HPLC) and the molecular weights determined using electrospray ionization mass-spectrometry.¹²

The new peptides have the following sequences:

H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-X⁹-Ser-Ala-Arg-X¹³-NH₂

Keywords: Opioid peptides; Nociceptin/orphanin FQ; NOP; Nociceptin analogues; SPPS; Rat vas deferens.

* Corresponding author. Tel.: +359 2 816 34 23; fax: +359 2 868 54 88; e-mail: e_naydenova@abv.bg

$X^{9,13}$ = Lys (1); X^9 = Lys, X^{13} = Orn (2); X^9 = Orn, X^{13} = Lys (3); $X^{9,13}$ = Orn (4); $X^{9,13}$ = Dab (5); $X^{9,13}$ = Dap (6).

The side chain of each consecutive compound is one methylene group shorter.



Lys, $R = (CH_2)_4NH_2$; Orn, $R = (CH_2)_3NH_2$; Dab, $R = (CH_2)_2NH_2$; Dap, $R = (CH_2)NH_2$

The biological activities of the parent compound and its analogues were tested in vitro on electrically stimulated rat vas deferens.¹³

Cumulatively administered in concentrations of 1×10^{-8} to 1×10^{-5} M, none of the newly synthesized N/OFQ-analogues markedly affected the tone of the isolated smooth muscles. The EC_{50} values and the E_{max} of the newly synthesized peptides on the LFES-evoked smooth-muscle contractions are presented in Table 1.

In order to elucidate the receptors involved in the smooth-muscle inhibitory effects of the new peptides, experiments were carried out with 1×10^{-6} M NAL that blocks the classical opioid—but not the NOP-receptors. In this experimental series, no significant changes in the inhibition of the smooth-muscle contractions by the tested peptides were registered (Figs. 1–4 and Table 1).

As shown on Table 1, the replacement of Lys with Orn at position 9 enhanced the inhibitory potency and the maximum effect of N/OFQ(1–13)NH₂. The same substitution at position 13 exerted an opposite effect on the biological activity of N/OFQ(1–13)NH₂. Therefore, it might be suggested that the side-chain reduction with one CH₂ group at position 9 resulted in increased inhibitory activity, whilst the same shortening of the side chain at position 13 led to a pronounced decrease in the inhibitory potency of the compound. Simultaneous substitution at positions 9 and 13 reduced the inhibitory effects of the parent compound on the LFES-induced contractions of rat vas deferens.

The present results revealed that the new N/OFQ-analogues might exert inhibitory effects on smooth-muscle contractions, and that this action probably did not involve classical opioid receptors.

Our results showed that the opioid antagonist naloxone did not change the strong inhibitory action of the newly

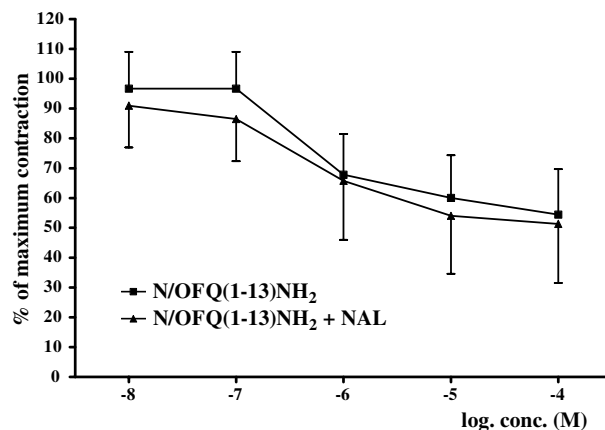


Figure 1. Rat vas deferens. Concentration–response curve of N/OFQ(1–13)NH₂ before and after naloxone, on the contractions induced by LFES. The data are means ± SEM of seven experiments.

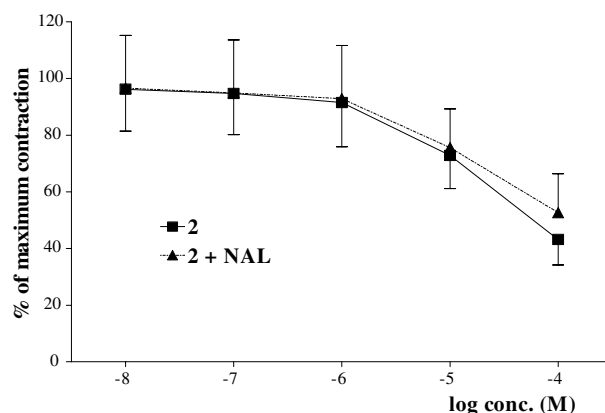


Figure 2. Rat vas deferens. Concentration–response curve of [Orn¹³]N/OFQ(1–13)NH₂ before and after naloxone, on the contractions induced by LFES. The data are means ± SEM of nine experiments.

synthesized N/OFQ(1–13)NH₂-analogues on LFES-evoked contractions. The data corroborate with the results obtained on the same tissue by Rizzi et al.¹⁴ It might be suggested, therefore, that classical opioid receptors were not involved in the inhibitory activity of the new N/OFQ-analogues. Most probably, their effects are due to interactions with NOP-receptors; since the changes in the N/OFQ(1–13)NH₂ molecule have been made in its ‘address’ part, the observed differences in the biological activity of the new compounds might be due to variations in their affinity towards NOP-receptors. Another possibility is that the peptides tested

Table 1. Effects and potency of the newly synthesized peptides on LFES-evoked contractions of rat vas deferens

Compound	Peptides tested	Peptide alone			NAL + peptide		
		pEC ₅₀	E _{max} (%)	Relative potency	pEC ₅₀	E _{max} (%)	Relative potency
1	N/OFQ(1–13)NH ₂	6.24 ± 0.30	–46 ± 15	1.00	6.40 ± 0.36	–49 ± 20	1.00
2	[Orn ¹³]N/OFQ(1–13)NH ₂	4.95 ± 0.14	–57 ± 9	0.05	4.96 ± 0.19	–49 ± 8	0.04
3	[Orn ⁹]N/OFQ(1–13)NH ₂	6.63 ± 0.20	–71 ± 7	2.45	6.67 ± 0.12	–77 ± 4	1.56
4	[Orn ^{9,13}]N/OFQ(1–13)NH ₂	6.39 ± 0.30	–56 ± 13	1.41	6.01 ± 0.27	–70 ± 9	2.45
5	[Dab ^{9,13}]N/OFQ(1–13)NH ₂	5.21 ± 0.19	–60 ± 12	0.09	5.03 ± 0.20	–33 ± 21	0.04
6	[Dap ^{9,13}]N/OFQ(1–13)NH ₂	5.63 ± 0.15	–54 ± 13	0.25	5.21 ± 0.17	–46 ± 12	0.06

Data represent mean values ± SEM of at least six separate experiments.

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