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[Nphe^1]nociceptin-(1–13)-NH_2 antagonizes nociceptin effects in the mouse colon

Anna Rizzi [a], Raffaella Bigoni [a], Girolamo Caló [a,*], Remo Guerrini [b], Severo Salvadori [b], Domenico Regoli [a]

[a] *Department of Experimental and Clinical Medicine, Section of Pharmacology, via Fossato Mortara, 17-19, Ferrara 44100, Italy*
[b] *Department of Pharmaceutical Sciences, University of Ferrara, via Fossato di Mortara, 17, Ferrara 44100, Italy*

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Abstract

Nociceptin, nociceptin-(1–13)-NH_2, Ac-RYYRWK-NH_2, [Phe$^1\psi$(CH$_2$—NH)Gly^2]nociceptin-(1–13)-NH_2, the new nociceptin analog [Nphe^1]nociceptin-(1–13)-NH_2, and endomorphin-1 have been tested in the isolated mouse colon. All peptides, except [Nphe^1]nociceptin-(1–13)-NH_2, caused concentration-dependent, tetrodotoxin-sensitive contractions showing similar maximal effects. Naloxone (1 μM) blocked the effect of endomorphin-1 but not that of the other peptides. [Nphe^1]nociceptin-(1–13)-NH_2 (10 μM) was inactive against endomorphin-1, but antagonized the contractile effects of nociceptin receptor ligands showing similar pA_2 values (≈ 6.0). The present findings indicate that [Nphe^1]nociceptin-(1–13)-NH_2 is a low-potency, selective nociceptin receptor antagonist, devoid of residual agonist activity.

Keywords: Nociceptin; Colon, mouse; [Nphe^1]nociceptin-(1–13)-NH_2

1. Introduction

Nociceptin/orphanin FQ is a newly discovered neuropeptide which activates a G-protein-coupled receptor (nociceptin receptor), structurally similar to opioid receptors (see Meunier, 1997 for a review). At the cellular level, activation of the nociceptin receptor inhibits cAMP accumulation and Ca^{2+} currents, while it stimulates K^+ conductance. As results of these cellular actions, nociceptin exerts mainly inhibitory effects on target cells: it inhibits the release of several neurotransmitters from brain preparations and neurogenic contractions induced by electrical stimulation in isolated tissues. These effects of nociceptin are resistant to the antagonistic action of the nonselective opioid receptor ligand, naloxone (Meunier, 1997). The role of nociceptin in the gastrointestinal system has been recently investigated (Osinski et al., 1999; Yazdani et al., 1999); in particular, it has been reported that nociceptin exerts a contractile action on mouse and rat colon in vitro.

The present study was undertaken to pharmacologically characterize nociceptin sites in the mouse colon; for this purpose, the effects of nociceptin, nociceptin-(1–13)-NH_2, Ac-RYYRWK-NH_2 (Dooley et al., 1997), [Phe$^1\psi$(CH$_2$—NH)Gly^2]nociceptin-(1–13)-NH_2 ([F/G]nociceptin-(1–13)-NH_2), and of the recently identified nociceptin receptor antagonist, [Nphe^1]nociceptin-(1–13)-NH_2 (Guerrini et al., 1999) were investigated in this preparation. In addition, the selectivity of nociceptin receptor ligands was determined by studying their effects in comparison with those exerted by the μ-opioid receptor ligands, endomorphin-1 and naloxone.

2. Experimental

For the experiments, segments of transverse and descending colon (1 cm in length) were obtained from male Swiss mice (25–30 g) and were prepared for recording of longitudinal isometric smooth muscle contraction, according to Osinski et al. (1999). Concentration–response curves to the compounds were performed noncumulatively, adding to the bath different concentrations of peptide every 20 min; antagonists were incubated for at least 15 min before

* Corresponding author. Tel.: +0039-532-291-221; fax: +0039-532-291-205.
E-mail address: g.calo@unife.it (G. Caló)

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Table 1
Antagonistic action of [Nphe^1]nociceptin-(1–13)-NH_2 against several nociceptin receptor ligands and endomorphin-1 in the isolated mouse colon
The data are mean of at least five separate experiments.
pEC_{50}: the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal effect.
E_{max}: the maximal effect induced by an agonist expressed as percentage of the contractile effect elicited by 10 μM carbachol (2.38 ± 0.44 g).
pA_2: the negative logarithm to base 10 of the antagonist molar concentration that makes it necessary to double the agonist concentration to elicit the original submaximal response. It has been calculated using the Gaddum–Schild equation: $pA_2 = \log((CR-1)/[antagonist])$.

	Control		+[Nphe^1]nociceptin-(1–13)-NH_2 10 μM		[Nphe^1]nociceptin-(1–13-NH_2
h3	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	pA_2
Nociceptin	48 ± 4	8.6	53 ± 9	7.5*	6.0
Nociceptin-(1–13)-NH_2	54 ± 8	8.6	50 ± 9	7.5*	6.0
[F/G]Nociceptin-(1–13)-NH_2	38 ± 4	7.9	38 ± 8	6.9*	5.9
Ac-RYYRWK-NH_2	48 ± 3	8.7	52 ± 4	7.5*	6.1
Endomorphin-1	42 ± 9	7.3	46 ± 7	7.2	inactive

* $p < 0.05$ vs. control according to the Student's *t*-test for paired data.

agonists. The peptides used in this study were prepared and purified as previously described (Calo et al., 1998; Guerrini et al., 1999). Naloxone and carbachol were from Sigma (St. Louis, USA). Nociceptin, nociceptin-(1–13)-NH_2, Ac-RYYRWK-NH_2, [F/G]nociceptin-(1–13)-NH_2, endomorphin-1, and carbachol elicited a concentration-dependent contraction of mouse colon tissues; [Nphe^1]nociceptin-(1–13)-NH_2 up to 10 μM was inactive. In the presence of tetrodotoxin (0.3 μM), which, per se, caused an increase in spontaneous contractions, the effects of the peptides were abolished while that of carbachol was still evident. Atropine (1 μM) prevented the effect of carbachol but not that of the peptides. These results are in line with those obtained by Yazdani et al. (1999) in the rat colon, and corroborate the view that nociceptin acts via a neuronal pathway not involving acetylcholine. The contractile action of nociceptin probably results from an inhibition of an inhibitory pathway within the myenteric plexus (Yazdani et al., 1999). Interestingly, in the mouse colon, [F/G]nociceptin-(1–13)-NH_2 acts as full agonist, confirming previous findings by Corbett et al. (1998). This pseudopeptide has been reported in several studies to act as an antagonist, partial agonist or even full agonist at nociceptin receptors depending on the preparations under study. The reason of this dual action of [F/G]nociceptin-(1–13)-NH_2 is at present unknown, but it is likely that the pseudopeptide is actually a low-efficacy agonist whose final effect depends on the stimulus–response efficiency of the preparation under study.

Naloxone (1 μM) does not modify the concentration–response curve to nociceptin receptor ligands while it fully prevents the contractile effect of endomorphin-1 (not shown). In contrast, [Nphe^1]nociceptin-(1–13)-NH_2 was found to be inactive against endomorphin-1, while it antagonizes the effects of nociceptin, nociceptin-(1–13)-NH_2, Ac-RYYRWK-NH_2 and [F/G]nociceptin-(1–13)-NH_2 with similar pA_2 values (Table 1). These results demonstrate that the contractile effect of these peptides is due to the activation of the nociceptin receptor and that [Nphe^1]nociceptin-(1–13)-NH_2 acts as a selective nociceptin receptor antagonist with low potency ($pA_2 \approx 6$). Similar pA_2 values were obtained for [Nphe^1]nociceptin-(1–13)-NH_2 in vitro using electrically stimulated tissues and in Chinese hamster ovary cells expressing the human recombinant nociceptin receptor (Calo et al., 1999). [Nphe^1]nociceptin-(1–13)-NH_2 is also active in vivo where it prevents the pronociceptive and antimorphine actions of i.c.v.-injected nociceptin in the mouse tail withdrawal assay (Calo et al., 1999).

3. Conclusion

The present data clearly demonstrate that [Nphe^1]nociceptin-(1–13)-NH_2 acts as a selective antagonist at nociceptin receptors mediating contraction of the mouse colon. Despite low potency, [Nphe^1]nociceptin-(1–13)-NH_2 is the first nociceptin antagonist devoid of residual agonistic activity. This pure and selective nociceptin receptor antagonist will be useful in future studies aimed to clarify the physiological and pathophysiological role of the nociceptin–nociceptin receptor system in central and peripheral tissues.

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