

Nociceptin/orphanin FQ inhibits capsaicin-induced guinea-pig airway contraction through an inward-rectifier potassium channel

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1 Nociceptin/orphanin FQ (N/OFQ), an endogenous opioid-like orphan receptor (NOP receptor, previously termed ORL1 receptor) agonist, has been found to inhibit capsaicin-induced bronchoconstriction in isolated guinea-pig lungs and *in vivo*. The underlying mechanisms are not clear. In the present studies, we tested the effect of N/OFQ on VR1 channel function in isolated guinea-pig nodose ganglia cells. Capsaicin increased intracellular Ca²⁺ concentration in these cells through activation of vanilloid receptors. Capsaicin-induced Ca²⁺ responses were attenuated by pretreatment of nodose neurons with N/OFQ (1 μM).

2 N/OFQ inhibitory effect on the Ca²⁺ response in nodose ganglia cells was antagonized by tertiapin (0.5 μM), an inhibitor of inward-rectifier K⁺ channels, but not by verapamil, a voltage gated Ca²⁺ channel blocker, indicating that an inward-rectifier K⁺ channel is involved in N/OFQ inhibitory effect.

3 In isolated guinea-pig bronchus, N/OFQ (1 μM) inhibited capsaicin-induced airway contraction. Tertiapin (0.5 μM) abolished the N/OFQ inhibition of capsaicin-induced bronchial contraction.

4 Capsaicin (10 μg) increased pulmonary inflation pressure in the isolated perfused guinea-pig lungs. This response was significantly attenuated by pretreatment with N/OFQ (1 μM). Tertiapin also abolished the N/OFQ inhibitory effect on capsaicin-induced bronchoconstriction in perfused lungs. Capsaicin increased the release of substance P and neurokinin A from isolated lungs. N/OFQ (1 μM) blocked the capsaicin-induced tachykinin release.

5 These results indicate that N/OFQ-induced hyperpolarization of tachykinin containing airway sensory nerves, through an inward-rectifier K⁺ channel activation, accounts for the inhibition of capsaicin-evoked bronchoconstriction.

British Journal of Pharmacology (2002) **135**, 764–770

Keywords: Capsaicin; vanilloid receptor; nociceptin; opioid-like orphan receptor; intracellular calcium; tachykinin; airway; nodose ganglion

Abbreviations: [Ca²⁺]_i, intracellular Ca²⁺; EFS, electrical field stimulation; FLIPR, fluorometric imaging plate reader; HBSS, Hank's balanced salt solution; NKA, neurokinin A; N/OFQ, nociceptin/orphanin FQ; NOP receptor, opioid-like orphan receptor; PIP, pulmonary inflation pressure; VR, vanilloid receptor

Introduction

Capsaicin-sensitive vanilloid receptor (VR) is expressed mainly in sensory neurons including those emanating from the nodose ganglion (visceral sensory neurons) and solitary tract region, the latter representing the central termination area for sensory neurons of the vagus nerve (Szallasi & Blumberg, 1999). VR has been detected in guinea-pig and human airways by receptor binding assay (Szallasi *et al.*, 1995). VR is a cation channel with preference for Ca²⁺. Activation of VR in sensory nerves by vanilloids such as capsaicin, induces Ca²⁺ influx and membrane depolarization, leading to release of neurotransmitters from both peripheral and central endings, resulting in neurogenic inflammation, bronchoconstriction, cough, and the sensation of pain. Included among these neurotransmitters are substance P (SP), neurokinin A (NKA), and calcitonin gene-

related peptide (CGRP, Holzer, 1988). In guinea-pig lungs, for example, capsaicin is known to induce bronchoconstriction mediated by a tachykinin-dependent mechanism, mainly through activation of NK₂ receptors (Lou *et al.*, 1993).

Opioid-like orphan receptors are widely expressed in mammalian central nervous system and several peripheral tissues including peripheral ganglia and the immune system (Mollereau & Mouldous, 2000). N/OFQ is an endogenous ligand for the NOP receptor (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). N/OFQ-containing nerve fibres have been detected in guinea-pig bronchus by immunohistochemical method (Fischer *et al.*, 1998). NOP receptor activation induces inhibition of voltage-gated Ca²⁺ channel current (Knoflach *et al.*, 1996; Abdulla & Smith, 1997; Connor & Christie, 1998; Moran *et al.*, 2000; Larsson *et al.*, 2000), and activation of inward-rectifier K⁺ channels in neurons (Lee *et al.*, 1997; Ikeda *et al.*, 1997; Wagner *et al.*, 1998), either of which may lead to inhibition of neurotransmitter release. The

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interaction between NOP and VR₁ receptors has presently not been studied.

In the airways, N/OFQ was found to inhibit the contractions of the guinea-pig isolated bronchus and tachykinin release induced by non-adrenergic non-cholinergic electrical field stimulation (EFS, Fischer *et al.*, 1998; Shah *et al.*, 1998). Recently, studies by Corboz *et al.* (2000; 2001) showed that N/OFQ also inhibited capsaicin-induced bronchoconstriction in isolated lungs and *in vivo* in guinea-pigs. We hypothesized that this effect of N/OFQ might be secondary to the inhibition of capsaicin-induced cell depolarization and tachykinin release from sensory neurons in the airway. In the current studies, we evaluated the interaction between NOP and VR₁ receptors at the cellular level using fluorescence-based calcium imaging experiments performed on isolated nodose ganglion neurons. We also tested the mechanism of N/OFQ inhibitory effect on capsaicin-induced airway contraction in isolated guinea-pig bronchus and in isolated perfused lungs.

Methods

Nodose ganglia cell isolation

Male Hartley guinea-pigs (400–700 g, Charles River, Bloomington, MA, U.S.A.) were euthanized with CO₂. Nodose ganglia were removed under aseptic conditions. Individual ganglia were collected, washed in Hank's balanced salt solution (HBSS) and then transferred to HBSS containing collagenase (type IA, 1 mg ml⁻¹) for 45 min at 37°C in a water bath. The enzyme solution was aspirated from the tissues, after which they were rinsed with HBSS and then incubated in HBSS containing DNase IV (0.1 mg ml⁻¹) for 15 min at 37°C in a water bath. Tissues were washed and subjected to gentle trituration using a Pasteur pipette. This was sufficient to dissociate the ganglia. The resulting cell suspension was filtered through a sterile nylon mesh (Becton Dickinson Labware MA, U.S.A.), plated into poly-lysine coated black walled clear-based 96-well plates (~10000 cells/well, Becton Dickinson Labware MA, U.S.A.). Cells were incubated for 3 h at 37°C prior to the intracellular Ca²⁺ measurements.

Measurements of intracellular Ca²⁺ concentration using the FLIPR

Intracellular Ca²⁺ ([Ca²⁺]_i) in nodose ganglia cells was measured using fluorometric imaging plate reader (FLIPR, Molecular Devices Corp., CA, U.S.A.) technique. Briefly, cells were incubated with a calcium sensitive fluoresce dye, Fluo-4-AM (5 µg ml⁻¹, Molecular Probes, OR, U.S.A.), in HBSS containing 0.4% bovine serum albumin (BSA) for 45 min at 37°C. The dye-loading solution was removed and the cells were washed twice with HBSS containing 0.4% BSA. The cells were preincubated with various antagonists and the plates were then placed into a FLIPR. Fluorescence change due to the change of [Ca²⁺]_i was measured by FLIPR. Capsaicin responses were elicited by direct additions to an individual culture well during real-time recording (10 s after commencing recording). We made no attempt to

calibrate the capsaicin response in terms of intracellular calcium concentration changes.

Isometric force measurement in isolated guinea-pig bronchus

Male Hartley guinea-pigs (400–700 g) were euthanized with CO₂ and their main bronchus were immediately excised and cut into rings. Only the rings from the upper end of the bronchus were used to measure the mechanical responses. Bronchial rings were mounted on hooks, connected to force transducers (model FT03, Grass, Quincy, MA, U.S.A.), and incubated in a physiological saline solution containing (in mM): NaCl 118, CaCl₂ 2.55, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24.9, Glucose 11.1 and indomethacin 2 µM (pH=7.4) bubbled with 95% O₂–5% CO₂ in 25-ml organ baths at 37°C. The passive tension was set at 1 g, and the tissue was equilibrated for 60 min in the presence of 1 µM phosphoramidon. Bronchus was then preincubated with or without tertiapin (40 min), verapamil (1 µM, 40 min) and/or N/OFQ (20 min). Isometric force of the bronchial rings in response to cumulative concentration of capsaicin was recorded. Only one concentration-response curve for capsaicin was performed in each bronchus.

Pulmonary inflation pressure in isolated guinea-pig lungs

The isolated lungs from guinea-pigs were used to test the effect of nociceptin on capsaicin-induced bronchoconstriction and tachykinin release. Male Hartley guinea-pigs (400–700 g), were euthanized with an intraperitoneal overdose of sodium pentobarbital. The lungs were isolated and perfused as described previously (Vemulapalli *et al.*, 1992). Briefly, a thoracotomy was rapidly performed and the lungs and the heart, with exception of the left atria, were removed *en bloc*. The trachea and pulmonary artery were rapidly cannulated. The lungs were then suspended inside a glass chamber (37°C) and perfused with a Tyrode's solution (mM: NaCl 137.0, KCl 2.7, CaCl₂ 0.4, MgCl₂·6H₂O 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.4 and dextrose 5.5) maintained at 37°C. They were mechanically ventilated with room air using a small rodent ventilator (Harvard). The respiratory rate was set at 60 strokes min⁻¹ with a volume of 2.0 ml stroke⁻¹. Pulmonary inflation pressure (PIP) was continuously monitored with a pressure transducer (Gould P231D) connected to a side arm of the tracheal cannula. Perfusion pressure was maintained with a peristaltic pump (Cole-Palmer 7553-20) at a rate of 4.5–5.0 ml min⁻¹ to produce a baseline pulmonary artery pressure between 6–14 cm H₂O. Pulmonary artery pressure was continuously monitored using a pressure transducer (Stathun P23XL) connected to the side arm of the pulmonary artery cannula. Transducers were connected to a polygraph (Grass Model no.7) for continuous monitoring of variables.

Tachykinin measurement

Lung perfusate was collected before and 6 min after capsaicin application. All the samples were collected for 3 min. Protease inhibitors (aprotonin 500 KIU ml⁻¹, captopril 1 µM, phosphoramidon 1 µM, bestanin 1 µM, and thiorphan 1 µM) were added to all samples immediately after sample collection. Samples were centrifuged at

17,000×*g* for 15 min at 4°C in the equal volume of 1% trifluoroacetic acid. Supernatant was passed through a 200 mg C18 column (Waters MA, U.S.A.) and eluted with 2 ml of solution containing acetonitrile (60%) and 1% TFA (40%). Eluent was dried using a speed vacuum system and reconstituted to a desired volume with assay buffer. SP and NKA levels were determined by enzyme immunoassay (ELISA) method using Enzyme Immunoassay Kit from Peninsula Laboratories Inc.

Data analysis

All results are expressed as means ± standard error of the mean (s.e.mean). An analysis of variance was performed on the different treatment groups to determine significant effects of the treatments. *Post-hoc* analysis between the different groups was performed with a Dunnett's *t*-test. Mann–Whitney Rank Sum Test was used to analyse non-normal distributed data (Figure 3B). A value of $P < 0.05$ was accepted as the level of statistical significance.

Materials

Capsaicin, tertiapin, DNase IV, collagenase (type IA), aprotinin, captopril, bestatin and thiorphan were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), nociceptin/orphanin FQ from Phoenix Pharmaceuticals (Mountain View, CA, U.S.A.), [Phe¹φ(CH₂-NH)Gly²]Nociceptin(1-13)NH from Tocris (Avonmouth Bristol, U.K.), Verapamil from ICN Biomedicals Inc. (Aroro, Ohio, U.S.A.), and substance P and NKA ELISA kits from Peninsula Labs (Belmont, CA, U.S.A.). HBSS was obtained from Gibco (NY, U.S.A.). Indomethacin was synthesized by Schering-Plough Corp.

Results

Ca²⁺ response in isolated guinea-pig nodose ganglia cells

Capsaicin (0.1 μM) consistently increased [Ca²⁺]_i in nodose ganglia cells as determined by Fluo-4 fluorescence change (Figure 1A). The concentration of capsaicin studied in these experiments was selected so as to be sub-maximal to avoid VR₁ receptor saturation. The capsaicin response was inhibited by 88 ± 6% when cells were pretreated with capsazepine (1 μM), a VR₁ antagonist (Figure 1A,C), indicating that the capsaicin-induced Ca²⁺ response was VR₁ receptor-mediated. Pre-treatment with N/OFQ (1 μM) attenuated the subsequent capsaicin-evoked response by 55 ± 5% (Figure 1B,C). This inhibitory effect was abolished by [Phe¹φ(CH₂-NH)Gly²]Nociceptin(1-13)NH, a NOP receptor antagonist (Figure 1C). The inhibitory effect of N/OFQ was also significantly antagonized (to 20 ± 4%) by prior blockade of inward rectifier potassium channels with tertiapin (0.5 μM, Jin & Lu, 1998; Kitamura *et al.*, 2000), whereas tertiapin itself had no effect on capsaicin-induced response (Figure 1B,C). Similar effects were also observed using single cell Fluo-4 imaging (unpublished observation). Verapamil, a voltage-gated Ca²⁺ channel blocker, had no effect on N/OFQ inhibitory effect (Figure 1C).

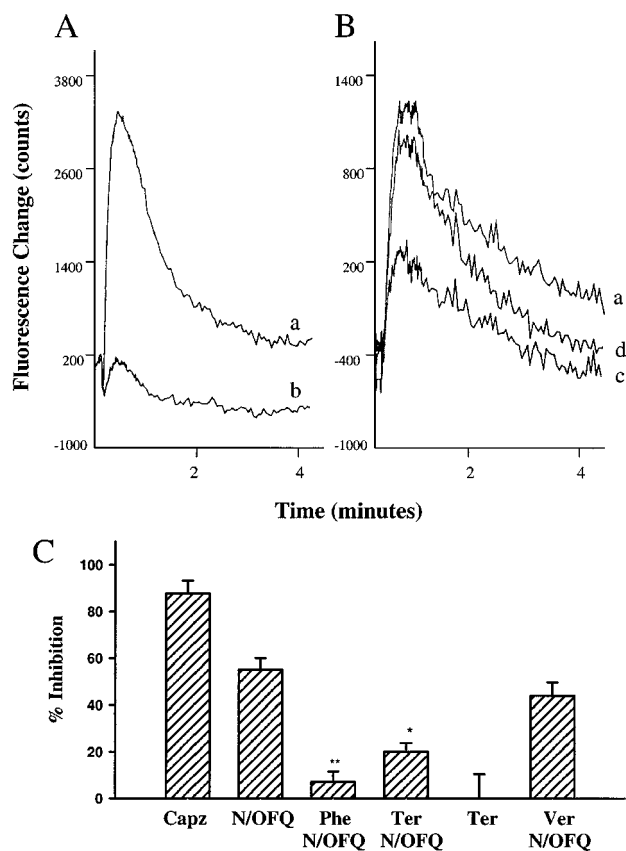


Figure 1 Effect of N/OFQ on capsaicin-induced [Ca²⁺]_i increase in guinea-pig nodose ganglia cells. [Ca²⁺]_i was determined by Fluo-4 fluorescence change and measured using FLIPR. Representative traces showing the Ca²⁺ response to capsaicin in nodose ganglia cells, in the presence of capsazepine (A), N/OFQ and tertiapin (B). Capsaicin 0.1 μM was added 10 s after commencing recording in all the experiments. a: control; b: cells preincubated with capsazepine (1 μM) for 10 min before the addition of capsaicin; c: cells pretreated with N/OFQ (1 μM) for 10 min before the addition of capsaicin; d: cells pretreated with tertiapin (0.5 μM) for 20 min and N/OFQ (1 μM) for 10 min before the addition of capsaicin. The recordings are representative examples from four experiments. (C) % inhibition of the capsaicin-induced fluorescence change by capsazepine (Capz, 1 μM), N/OFQ (1 μM), [Phe¹φ(CH₂-NH)Gly²]Nociceptin(1-13)NH (Phe, 10 μM)+N/OFQ (1 μM), tertiapin (Ter, 0.5 μM)+N/OFQ (1 μM), Tertiapin (0.5 μM), and verapamil (Ver, 1 μM)+N/OFQ (1 μM, $P = 0.13$ compared with N/OFQ group). Data are means ± s.e.mean. * $P < 0.001$, ** $P < 0.0001$ compared with N/OFQ treatment.

Effect of tertiapin on N/OFQ inhibition of capsaicin response in isolated guinea-pig bronchus

Figure 2A shows the dose response curves of the capsaicin-induced bronchocontraction in the isolated guinea-pig bronchus. The response was expressed as the percentage of the maximal response induced by KCl (80 mM). N/OFQ (1 μM), when preincubated with bronchus, significantly inhibited the capsaicin-induced airway contraction. In tissues preincubated with tertiapin (0.5 μM), the N/OFQ inhibitory effect on the capsaicin-induced bronchocontraction was completely abolished. Tertiapin alone had no effect on capsaicin-induced response in isolated bronchus (Figure 2A). N/OFQ inhibitory effect was not affected by verapamil (1 μM, Figure 2B).

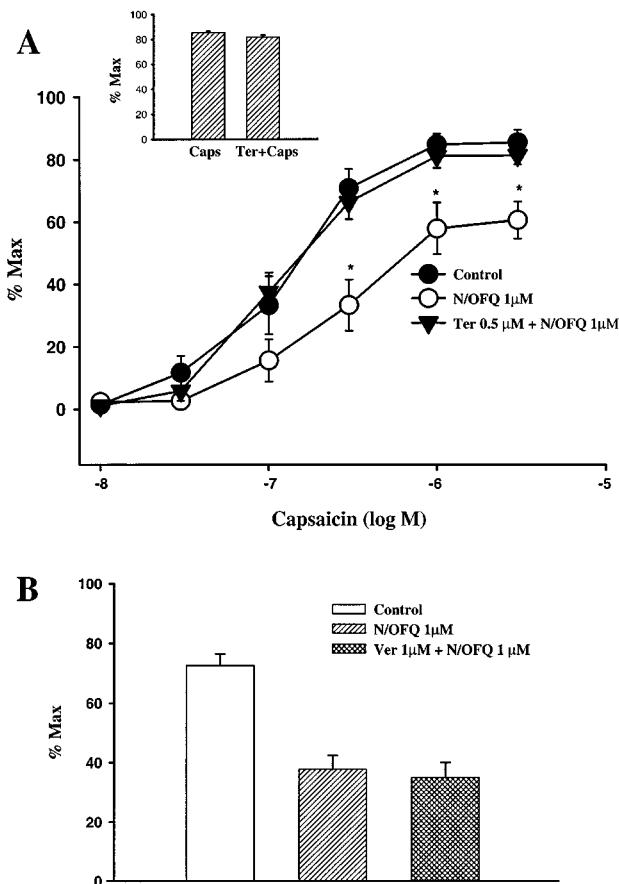


Figure 2 (A) Effect of tertiapin on N/OFQ inhibition of the capsaicin response in isolated guinea-pig bronchus. The results are means \pm s.e.mean and are expressed as percentage of the maximal response induced by KCl (80 mM). In this experiment, N/OFQ and tertiapin were always applied 20 min and 40 min, respectively, before the addition of capsaicin. * $P < 0.05$ as compared with control or N/OFQ + tertiapin treatments. Insert bars: Effect of tertiapin (Ter, 0.5 μ M) on capsaicin (1 μ M)-induced contraction ($n = 7$). (B) Lack of effect of verapamil (Ver, $n = 6$) on N/OFQ inhibition of capsaicin (30 nM) response in isolated guinea-pig bronchus.

Effects of N/OFQ on capsaicin-induced bronchoconstriction in isolated guinea-pig lungs

The bronchoconstrictor response to capsaicin was measured in isolated guinea-pig lungs in the presence and absence of N/OFQ (Figure 3A). The PIP baseline was 13.4 ± 0.5 mmHg in control group ($n = 6$) and 12.0 ± 0.5 mmHg in N/OFQ (1 μ M) pretreatment group ($n = 6$). Bolus injection of capsaicin (10 μ g in 100 μ l) to the pulmonary artery significantly increased PIP in both control (47.8 ± 5.1 mmHg, $P < 0.01$) and N/OFQ (29.8 ± 2.3 mmHg, $P < 0.005$) groups. However, the peak PIP response to capsaicin in N/OFQ group was significantly lower than that in control group ($P < 0.005$, Figure 3A). Tertiapin (0.5 μ M) reversed the N/OFQ inhibitory effect, but had no direct effect on capsaicin-induced bronchoconstriction response (Figure 3A).

Effect of N/OFQ on capsaicin-induced NKA and substance P release from isolated guinea-pig lungs

NKA and substance P concentrations in lung perfusate were measured before and after bolus injection of capsaicin.

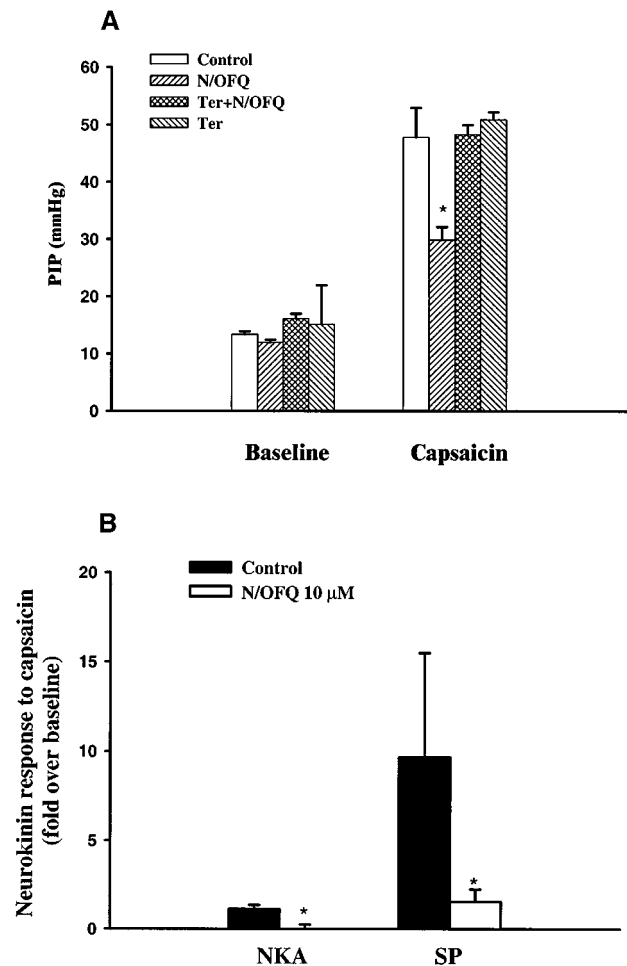


Figure 3 Effect of N/OFQ on capsaicin-induced bronchoconstriction and neurokinin release in isolated guinea-pig lungs. (A) The PIP response to capsaicin was measured in isolated perfused guinea-pig lungs. The results are means \pm s.e.mean. * $P < 0.005$ as compared with the control group in the absence of nociceptin. (B) Lung perfusate was collected before and after the bolus injection of capsaicin (10 μ g). NKA ($n = 6$) and substance P ($n = 5$) concentration in lung perfusate was measured. Capsaicin responses were expressed as fold over baseline. The results are means \pm s.e.mean. * $P < 0.05$ as compared with N/OFQ group.

Capsaicin induced a significant increase in tachykinin levels in the lung perfusate. NKA and substance P levels were increased by 2.1 ± 0.3 and 10.7 ± 5.8 fold of baseline respectively ($P < 0.05$, Figure 3B). N/OFQ (1 μ M, 20 min) induced a statistically significant inhibition of this stimulatory effect of capsaicin ($P < 0.05$, Figure 3B). N/OFQ had no direct effect on baseline tachykinin release (data not shown).

Discussion

We recently found that N/OFQ inhibited capsaicin-induced bronchoconstriction in isolated guinea-pig lungs (Corboz *et al.*, 2000) and *in vivo* (Corboz *et al.*, 2001). The mechanism has not been previously elucidated. Clearly, a plausible mechanism might involve a direct NOP receptor-mediated inhibition of VR1 calcium influx, and/or an indirect effect *via* membrane hyperpolarization of sensory nerve terminals,

leading to a decrease of tachykinin release. In the current studies, we tested the effect of N/OFQ on VR₁ function in isolated guinea-pig nodose ganglia cells. We found that N/OFQ inhibited capsaicin-induced responses in guinea-pig nodose ganglia cells as well as in isolated bronchus and lungs. N/OFQ inhibitory effects were antagonized by the inward-rectifier potassium channel antagonist, tertiapin.

The nodose ganglion is a sensory ganglion whose axons run in the vagus nerve (vagal afferents) and provide sensory innervation to the viscera including airways. VR₁ receptor has been detected using *in situ* hybridization (Michael & Priestley, 1999) and functionally (Bielefeldt, 2000) in nodose ganglia neurons. Our findings showing that capsaicin-induced Ca²⁺ responses in isolated guinea-pig nodose ganglia cells are blocked by capsazepine, a VR₁ antagonist, confirmed the functional expression of VR₁ receptors in these cells. In this study, we also found that N/OFQ inhibited capsaicin-induced increase in [Ca²⁺]_i in nodose ganglia cells *via* an action on NOP receptors because the inhibitory effect of N/OFQ was antagonized by [Phe¹φ(CH₂-NH)Gly²]Nociceptin(1-13)NH, an NOP receptor antagonist. These observations are consistent with N/OFQ's effect on airway contraction *in vitro* and *in vivo* (Corboz *et al.*, 2000; 2001). This finding also indicates that NOP receptors are functionally expressed in nodose ganglia neurons. Thus, these cells were ideal for the study of the mechanism by which N/OFQ inhibits VR₁ function in the airways.

A prime candidate for the mechanism underlying the NOP/VR₁ receptor interaction may be a change in membrane polarization. N/OFQ has been reported to produce a membrane hyperpolarization of β-endorphin neurons and other neurosecretory cells in the hypothalamic arcuate nucleus by activation of an inward-rectifier K⁺ conductance (Lee *et al.*, 1997; Wagner *et al.*, 1998). N/OFQ also induces hyperpolarizing currents *via* an inward-rectifier K⁺ channel in hippocampal pyramidal cells (Ikeda *et al.*, 1997). To test whether these mechanisms are involved in the N/OFQ inhibition of bronchoconstriction induced by capsaicin, we tested the effect of tertiapin, a novel inhibitor for inward-rectifier K⁺ channels (Jin & Lu, 1998; Kitamura *et al.*, 2000), on the N/OFQ inhibitory effect. The N/OFQ inhibitory effect on capsaicin-induced Ca²⁺ influx in guinea-pig nodose ganglia cells was reversed by tertiapin (Figure 1B,C). Consistently, N/OFQ's inhibition of capsaicin-induced bronchial contraction in isolated bronchus and perfused lungs were also abolished by tertiapin (Figures 2A, 3A). These results indicate that an inward-rectifier K⁺ channel is involved in the N/OFQ's inhibitory action. Activation of a K⁺ conductance by N/OFQ would lead to membrane hyperpolarization, a concomitant inhibition of neuronal firing and, presumably, attenuated neurotransmitter release. Indeed, we found that N/OFQ inhibited capsaicin-induced substance P and NKA release from isolated guinea-pig lungs, consistent with this proposed mechanism. Similar observations have also been reported in rats that N/OFQ inhibits capsaicin-induced SP and CGRP release in rat trachea (Nemeth *et al.*, 1998).

N/OFQ is also known to inhibit voltage-dependent Ca²⁺ channels in several neurons (Knoflach *et al.*, 1996; Abdulla *et al.*, 1997; Connor & Christie, 1998; Moran *et al.*, 2000; Larsson *et al.*, 2000). We considered the possibility that inhibition of voltage-dependent Ca²⁺ channels was also

involved in the observed N/OFQ inhibitory effect, though this would be unlikely in view of the known capsaicin activation of Ca²⁺ influx via the VR₁ cation channel. Accordingly, we found that blockade of voltage-activated Ca²⁺ channels by verapamil did not affect the N/OFQ inhibitory effect in either nodose ganglia cells or isolated bronchus. Likewise, capsaicin-induced SP release from rat trachea is independent of voltage-dependent Ca²⁺ channels (Szolcsanyi *et al.*, 1998).

NOP receptors have been shown to inhibit neurotransmitter release from both central and peripheral neurons. In the respiratory system, N/OFQ has been shown to inhibit electrical field stimulation (EFS)-induced acetylcholine release from parasympathetic nerve terminals in guinea-pig trachea (Patel *et al.*, 1997). EFS of guinea-pig isolated main bronchi induced a nonadrenergic non-cholinergic contractile response and release of substance P in guinea-pig airway (Fischer *et al.*, 1998; Shah *et al.*, 1998). N/OFQ inhibits the EFS induced bronchi contraction and substance P release (Shah *et al.*, 1998). N/OFQ also inhibits EFS-induced substance P and CGRP release from the rat isolated trachea (Helyes *et al.*, 1997; Shah *et al.*, 1998). Our current findings provide the first evidence that N/OFQ also inhibits the tachykinin release in the guinea-pig lungs evoked by capsaicin. It is possible that the same mechanism involving the activation of inward-rectifier K⁺ channels also accounts for the N/OFQ inhibition of neurotransmitter release from central and other peripheral systems, as these channels are widely expressed in neurosecretory cells.

Although peptide ligands are generally highly specific for their respective receptors, we considered the remote possibility that inhibition of capsaicin-induced tachykinin release by N/OFQ may also involve a direct effect of the peptide on the VR₁ channel. However, the present tertiapin data argue against this being the case, favouring membrane hyperpolarization as the causative mechanism linking the two receptor systems. Indeed, recent experiments using cloned rat VR₁ receptors have demonstrated their sensitivity to membrane hyperpolarization (Gunthorpe *et al.*, 2000). Current-voltage relationships for recombinant VR₁ showed a region of negative-slope conductance consistent with channel function being compromised by membrane potentials more negative to -70 mV (Gunthorpe *et al.*, 2000). These findings are consistent with the mechanism that N/OFQ inhibits capsaicin-induced VR₁ activation through a hyperpolarizing K⁺ current.

In this study, we found that N/OFQ (1 μM) inhibited capsaicin-induced airway contraction in isolated guinea-pig bronchus. This finding is consistent with previous reports in perfused lungs (Corboz *et al.*, 2000) and *in vivo* (Corboz *et al.*, 2001). However, other groups reported that in the presence of N/OFQ, capsaicin-induced contraction in isolated guinea-pig bronchus was not affected (Fischer *et al.*, 1998; Shah *et al.*, 1998). In the present study, the isolated bronchus was preincubated with N/OFQ for 20 min before the addition of capsaicin, this may be important for N/OFQ inhibitory effect and account for the difference from other reports. In addition, in the study by Fischer *et al.* (1998), a 10 fold lower concentration of the peptide was used while in the study by Shah *et al.* (1998), (where the same concentration of N/OFQ was tested), a clear trend toward a peptide induced inhibitory effect was evident, although it did not reach the statistical

level of significance. Therefore, we suggest that different experimental conditions and/or different assay sensitivities among laboratories may account for the apparent discrepancies.

The functional role of NOP activation on sensory airway reflexes has been recently investigated. Recent studies have been shown that selective activation of NOP receptors with N/OFQ produces antitussive activity in guinea-pigs (McLeod *et al.*, 2001) and cats (Bolser *et al.*, 2001). N/OFQ, when given by either a central or peripheral route, was found to inhibit capsaicin-induced cough in guinea-pigs. N/OFQ also inhibited cough induced by mechanical stimulation in cats. These findings are consistent with our current finding that NOP receptors are functional modulators of airway sensory responses. We propose that the antitussive activity of N/OFQ may also involve hyperpolarization of sensory neurons by activation of potassium channels.

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The authors are grateful to Dr R.L. McLeod, Dr. X-W Dong and Mr J. Crona for expert technical assistance and advice during the course of these experiments.

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(Received May 8, 2001
Revised October 2, 2001
Accepted November 16, 2001)