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Activation of the nociceptin/orphanin FQ receptor reduces bronchoconstriction and microvascular leakage in a rabbit model of gastroesophageal reflux

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1 Nociceptin/orphanin FQ (N/OFQ) is the endogenous peptide ligand for a specific G-protein coupled receptor, the N/OFQ peptide receptor (NOP). The N/OFQ-NOP receptor system has been reported to play an important role in pain, anxiety and appetite regulation. In airways, N/OFQ was found to inhibit the release of tachykinins and the bronchoconstriction and cough provoked by capsaicin.

2 Here we evaluated the effects of NOP receptor activation in bronchoconstriction and airway microvascular leakage induced by intraesophageal (i.oe.) hydrochloric acid (HCl) instillation in rabbits. We also tested the effects of NOP receptor activation in SP-induced plasma extravasation and bronchoconstriction.

3 In anesthetized New Zealand rabbits bronchopulmonary function (total lung resistance (R_L) and dynamic compliance (C_{dyn})) and airway microvascular leakage (extravasation of Evans blue dye) were evaluated.

4 Infusion of i.oe. HCl (1 N) led to a significant increase in bronchoconstriction and plasma extravasation in the main bronchi and trachea of rabbits pretreated with propranolol, atropine and phosphoramidon.

5 Bronchoconstriction and airway microvascular leakage were inhibited by N/OFQ (3–30 $\mu g\,kg^{-1}$ i.v.) in a dose-dependent manner. The NOP receptor agonist [Arg^{14},Lys^{15}]N/OFQ mimicked the inhibitory effect of N/OFQ, being 10-fold more potent, UFP-101, a peptide selective NOP receptor antagonist, blocked the inhibitory effects of both agonists.

6 Under the same experimental conditions, N/OFQ and [Arg^{14},Lys^{15}]N/OFQ did not counteract the bronchoconstriction and airway microvascular leakage induced by substance P.

7 These results suggest that bronchoconstriction and airway plasma extravasation induced by i.oe. HCl instillation are inhibited by activation of prejunctional NOP receptors.
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Abbreviations: C_{dyn}, dynamic compliance; CNS, central nervous system; GER, gastroesophageal reflux; HCl, hydrochloric acid; N/OFQ, nociceptin/orphanin FQ; NOP, N/OFQ peptide receptor; NOP-SA, selective agonist of NOP receptor: [Arg^{14},Lys^{15}]N/OFQ; NTS, nucleus of the solitary tract; R_L, total lung resistance; SP, substance P

Introduction

Nociceptin/orphanin FQ (N/OFQ) (Meunier *et al*., 1995; Reinscheid *et al*., 1995) is the endogenous peptide ligand for a specific opioid like G-protein coupled receptor recently named N/OFQ peptide receptor (NOP) (Cox *et al*., 2000). The N/OFQ-NOP receptor system has been reported to play an important role in various central functions such as pain, anxiety, memory, locomotion and appetite regulation as well as in the periphery on the cardiovascular, renal, gastrointestinal and airway systems (Calò *et al*., 2000; Mogil & Pasternak, 2001). NOP receptors are located both in the CNS and in the periphery, and mRNA is expressed in upper vagal sensory ganglia, where cell bodies of the tachykinin-containing sensory neurons are located (Peiser *et al*., 2000). In addition, N/OFQ-immunoreactive nerve fibers in the airway wall, distinct from the tachykinin-containing fibers, were identified as a pulmonary source of N/OFQ (Fischer *et al*., 1998; Peiser *et al*., 2000).

Recent data demonstrated that in the CNS, activation of NOP receptors by N/OFQ inhibits the release of several neurotransmitters such as noradrenaline, serotonin, acetylcho-

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line and glutamate (Schlicker & Morari, 2000). In airways, N/OFQ was found to inhibit the contractions of the guinea-pig isolated bronchus induced by electrical field stimulation (Fischer *et al*., 1998; Shah *et al*., 1998; Rizzi *et al*., 1999), an effect that is mediated by a prejunctional mechanism and not involving the classical opioid receptors. Other studies showed that N/OFQ inhibits acetylcholine release in the trachea (Patel *et al*., 1997) and capsaicin-induced bronchoconstriction in isolated lungs (Corboz *et al*., 2000). *In vivo* studies have shown that N/OFQ inhibits bronchoconstriction and cough induced by capsaicin in guinea-pigs (McLeod *et al*., 2001; Jia *et al*., 2002) or by mechanical stimulation of intrathoracic airways in the cat (Bolser *et al*., 2001). Finally, N/OFQ inhibits guinea-pig airway inflammation induced by esophageal hydrochloric acid (HCl) instillation (Rouget *et al*., 2004). Collectively, these studies demonstrate that N/OFQ may influence airway physiology by modulating cholinergic and/or tachykininergic neurotransmission.

Gastroesophageal reflux (GER) disease is a common condition contributing to cough, bronchoconstriction and airway inflammation (Theodoropoulos *et al*., 2002), and is frequent in asthmatic patients (Harding *et al*., 2000). Several lines of evidence suggest that GER may induce moderate bronchoconstriction in dogs (Mansfield *et al*., 1981; Ishikawa *et al*., 1999), rabbits (Gallelli *et al*., 2003), and in asthmatic patients (Spaulding *et al*., 1982; Hervè *et al*., 1986). Esophageal HCl perfusion also induced airway plasma extravasation in guinea-pigs (Hamamoto *et al*., 1997; Daoui *et al*., 2002). The mechanism by which GER might aggravate asthmatic symptoms remains unclear. Two major mechanisms have been proposed: (i) microaspiration of acid contents into the airways (reflux theory) (Ishikawa *et al*., 1999; Ricciardolo, 2001); (ii) involvement of vagal and sensory nerve terminals in the lower esophagus (reflex theory) (Mansfield & Stein, 1978; Kjellen *et al*., 1981; Hervè *et al*., 1986).

Although instillation of acid into airways could induce bronchoconstriction in animal models, pulmonary regurgitation of refluxed stomach contents, has not been clearly demonstrated in asthmatic patients with GER (Ghaed & Stein, 1979; Berquist *et al*., 1981). In contrast, the involvement of the vagus nerve and cholinergic neurotransmission in GER induced bronchoconstriction has been demonstrated in both animal and human studies (Mansfield *et al*., 1981; Hervè *et al*., 1986; Colson *et al*., 1990; Advenier *et al*., 2002).

The role of sensory nerves and of tachykinins in the airway effects of intraesophageal (i.oe.) HCl infusion has been demonstrated in animals. Indeed, airway plasma extravasation or bronchoconstriction (increase in total lung resistance (R_L) and decrease in dynamic compliance (C_{dyn})) induced by i.oe. HCl infusion in guinea-pigs (Hamamoto *et al*., 1997; Daoui *et al*., 2002) or rabbits (Daoui *et al*., 2002; Gallelli *et al*., 2003) is abolished by pretreatment with capsaicin, a drug that induces a depletion of tachykinins from sensory nerves or by treatment with neurokinin receptor antagonists (Hamamoto *et al*., 1997; Daoui *et al*., 2002; Gallelli *et al*., 2003). Tachykinin involvement in bronchoconstriction and airway microvascular leakage has been demonstrated with tachykinin receptor antagonists in our previous paper using this experimental model of GER (Gallelli *et al*., 2003).

Therefore, the aim of the present study was to evaluate in the rabbit the effects of N/OFQ in airway microvascular leakage and bronchoconstriction induced by i.oe. HCl instillation. We also tested the effects of recently described selective and potent NOP receptor agonist, [Arg^{14},Lys^{15}] N/OFQ (Okada *et al*., 2000; Rizzi *et al*., 2002), and antagonist UFP-101 (Calò *et al*., 2002), in this experimental model of GER.

Methods

Animals

Rabbits (2.5–2.8 kg) of either sex were used throughout the study. Animals were housed at constant temperature ($21 \pm 1^{\circ}C$), relative humidity ($55 \pm 5\%$), under a regular light–dark schedule (light 0700–1900). Food and water were freely available. The experimental procedures were in accordance with Italian DL. 116/92.

Bronchopulmonary function measurement

Animals were subjected to neuroleptoanalgesia (hypnorm 0.4 ml kg^{-1}; a mixture of fentanyl citrate 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1}, i.m.) and intubated with endotracheal and endoesophageal tubes for the measurement of flow and pleural pressure. Total lung resistance R_L and C_{dyn} values were evaluated through an on-line respiratory analyzer (PMS version 8.4, Mumed Ltd, London).

Esophageal stimulation with HCl

Following neuroleptoanalgesia, the thoracic wall was partly sectioned at the level of the 3rd–5th tracheal cartilage ring and a catheter placed in the midesophagus for HCl infusion. The upper portion of the esophagus was ligated to inhibit HCl leakage, while a latex balloon attached at the end of endoesophageal tube for pleural pressure, blocked all communication between esophagus and stomach.

Measurement of airway microvascular leakage

Vascular permeability was quantified by the extravasation of Evans blue dye (Rogers *et al*., 1988). Evans blue dye (30 mg kg^{-1}) was injected into the rabbit's ear marginal vein followed 1-min later by i.oe. HCl 1 N or saline (0.4 ml). After the induction of leakage (10 min after completion of the i.oe. HCl infusion), the thorax was opened and a blunt ended 13-14-gauge needle was passed through a left ventriculotomy into the aorta. The ventricule was cross-clamped and blood was expelled through an incision into the right atrium at 80 mmHg pressure with 700 ml of phosphate buffer. The lung was then removed and dye concentration was evaluated as previously described (Daoui *et al*., 2002).

Experimental protocol

All animals were pretreated 30 min before experimentation with atropine (1 mg kg^{-1}, i.p.) and propranolol (1 mg kg^{-1}, i.p.) to block muscarinic and beta-adrenergic receptors, and with phospharamidon (1 mg kg^{-1}, i.p.) to inhibit tachykinin degradation. To evaluate the effect of NOP receptor activation on bronchoconstriction and airway plasma extravasation, N/OFQ (3, 10 and 30 μg kg^{-1}) or [Arg^{14},Lys^{15}]N/OFQ (0.3

and 3 μg kg^{-1}), an NOP receptor agonist, or their saline vehicle were injected 1 min before Evans blue.

In a complementary series of experiments, UFP-101, an NOP receptor antagonist, or its vehicle was injected at the dose of 100 μg kg^{-1} 10 min before NOP receptor agonist injection. In a separate set of experiments, NOP receptor activation on SP-induced bronchoconstriction and airway plasma extravasation was evaluated. SP (0.3 μg kg^{-1}) was injected intravenously (i.v.) 5 min before Evans blue dye injection. Bronchoconstriction and leakage were evaluated 10 min later. N/OFQ was used at a dose of 30 μg kg^{-1} and [Arg^{14},Lys^{15}]N/OFQ at a dose of 3 μg kg^{-1}.

Drugs

Atropine sulfate, Evans blue dye, formamide, phosphoramidon, propranolol, HCl and SP were obtained from Sigma, Italy. Hypnorm was obtained from Janssen Pharmaceutical Ltd, Grove, Oxfordshire, U.K. [Arg^{14},Lys^{15}]N/OFQ, UFP-101 and N/OFQ were synthesized according to Guerrini *et al.* (1997). All the drugs used were dissolved in 0.9% NaCl.

Statistical analysis

All data are expressed as mean ± standard error (s.e.m.). Statistical evaluation was performed by analysis of variance (ANOVA), followed by the Student–Newman–Keuls post-test. The threshold of statistical significance was set at $P<0.05$.

Results

Effect of NOP receptor activation on HCl-induced bronchoconstriction

i.oe. HCl (1 N) infusion induced significant bronchoconstriction ($P<0.01$), in terms of R_L increase (Figure 1) and C_{dyn} decrease (data not shown). This effect was dose-dependently decreased by N/OFQ treatment ($P<0.01$) (Figure 1). Similar results were obtained using [Arg^{14},Lys^{15}]N/OFQ, an NOP receptor agonist (Figure 2); however, the N/OFQ analog was found to be approx 10-fold more potent than the natural peptide. Pretreatment with the selective NOP receptor antagonist (UFP-101; 100 μg kg^{-1}, i.v.) alone did not affect HCl induced bronchoconstriction, but blocked the effects of both N/OFQ and [Arg^{14},Lys^{15}]N/OFQ (Figures 1 and 2).

Effect of NOP receptor activation on HCl-induced plasma extravasation

i.oe. HCl (1 N) infusion significantly increased plasma extravasation in the trachea and main bronchi. N/OFQ produced a significant, dose-dependent decrease of HCl on microvascular leakage in both trachea and main bronchi (Figure 3). Treatment with [Arg^{14},Lys^{15}]N/OFQ (3 μg kg^{-1}, i.v.) produced a similar inhibitory effect to that of 30 μg kg^{-1}, i.v. N/OFQ (Figure 4). The NOP receptor antagonist UFP-101 (100 μg kg^{-1}, i.v.) alone had no effect on HCl-induced microvascular leakage, but blocked the inhibitory effects of both NOP agonists (Figures 3 and 4).

Effect of NOP receptor activation on SP-induced bronchoconstriction and airway microvascular leakage

SP (0.3 μg kg^{-1} i.v.) administration induced a significant bronchoconstriction ($P<0.01$), in terms of R_L increase (Figure 5) and C_{dyn} decrease (data not shown), and significantly increased plasma extravasation in the trachea and main bronchi (Figure 6). Both N/OFQ and [Arg^{14},Lys^{15}]N/OFQ, at doses active against i.oe. HCl infusion, did not produce any effect on SP-induced bronchoconstriction or plasma extravasation (Figures 5 and 6).

Discussion

In the present study we have documented the effects of NOP receptor activation on lung responses in the rabbit induced by i.oe. HCl infusion. In addition to our previous findings in the guinea-pig (Rouget *et al.*, 2004), we have shown that HCl induced plasma extravasation but also bronchoconstriction were significantly reduced by a pretreatment with N/OFQ

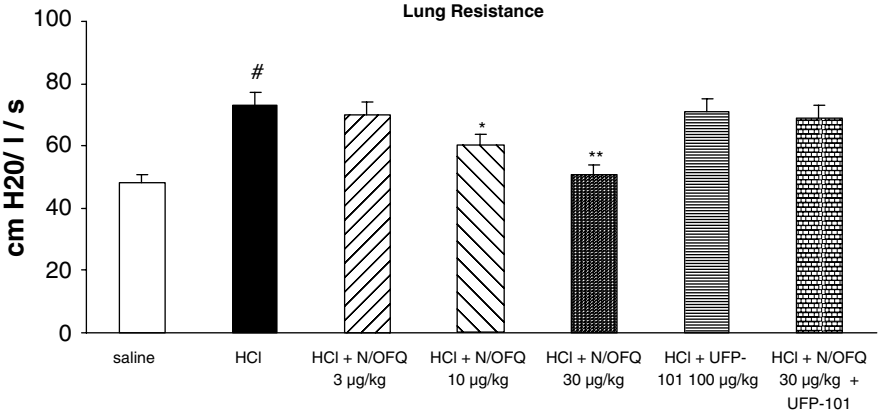

Figure 1 Effects of N/OFQ (3, 10 and 30 μg kg^{-1}) on bronchoconstriction induced by i.oe. HCl infusion (1 N) in anesthetized rabbits with or without NOP receptor antagonist (UFP-101) pretreatment. Rabbits were pretreated with atropine (1 mg kg^{-1}), propranolol (1 mg kg^{-1}) and phosphoramidon (1 mg kg^{-1}). UFP-101 was administered 10 min before agonists. All values are expressed as mean ± s.e.m., $n=5$ per group; $^{\#}P<0.01$ vs saline; $^{*}P<0.05$ vs HCl; $^{**}P<0.01$ vs HCl.

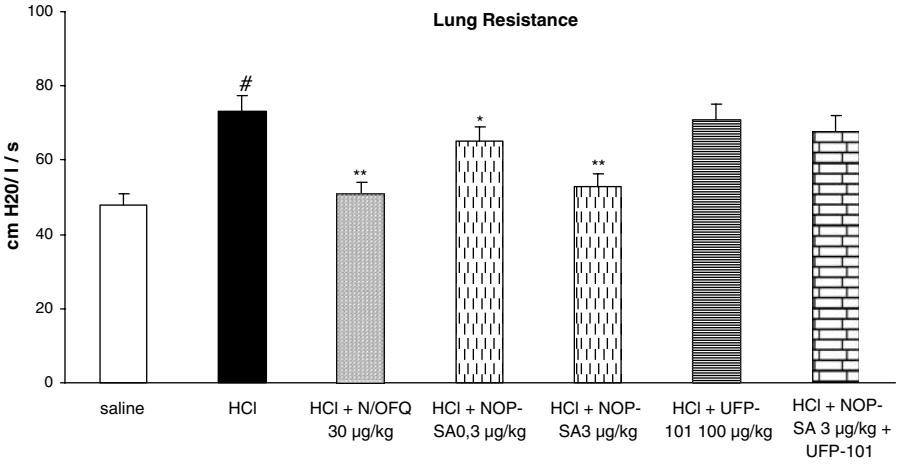

Figure 2 Comparison of the effects of NOP-SA (selective agonist of NOP receptor: [Arg^{14},Lys^{15}]N/OFQ) with respect to N/OFQ (30 μg kg^{-1}) on bronchoconstriction induced by i.oe. HCl infusion (1 N) in anesthetized rabbits with or without NOP receptor antagonist (UFP-101) pretreatment. Rabbits were pretreated with atropine (1 mg kg^{-1}), propranolol (1 mg kg^{-1}) and phosphoramidon (1 mg kg^{-1}). UFP-101 was administered 10 min before agonists. All values are expressed as mean $\pm$ s.e.m., $n=5$ per group. $^{\#}P<0.01$ vs saline; $^{*}P<0.05$ vs HCl; $^{**}P<0.01$ vs HCl.

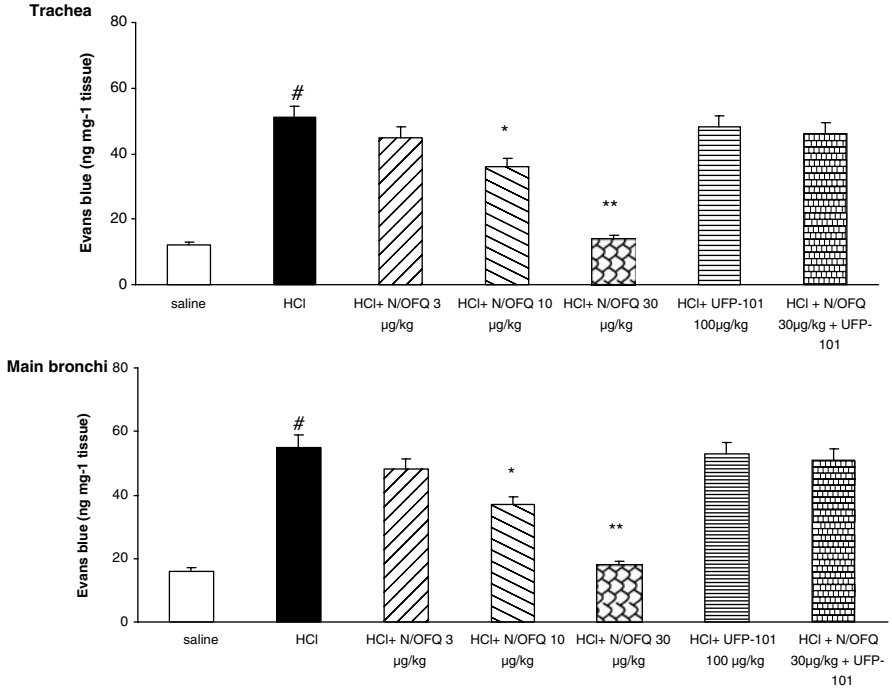

Figure 3 Effects of N/OFQ (3, 10 and 30 μg kg^{-1}) on the microvascular leakage induced by i.oe. HCl infusion (1 N) in anesthetized rabbits with or without NOP receptor antagonist (UFP-101) pretreatment. Rabbits were pretreated with atropine (1 mg kg^{-1}), propranolol (1 mg kg^{-1}) and phosphoramidon (1 mg kg^{-1}). UFP-101 was administered 10 min before agonists. All values are expressed as mean $\pm$ s.e.m., $n=5$ per group. $^{\#}P<0.01$ vs saline; $^{*}P<0.05$ vs HCl; $^{**}P<0.01$ vs HCl.

(10–30 μg kg^{-1}) and the highly potent NOP receptor agonist [Arg^{14},Lys^{15}]N/OFQ (0.3–3 μg kg^{-1}) (Okada *et al*., 2000; Rizzi *et al*., 2002). We have also shown that the effects of both agonists were abolished by the selective NOP receptor antagonist UFP-101 (Calò *et al*., 2002), suggesting that their preventive actions on vascular leakage and bronchoconstriction are exclusively due to NOP receptor activation and share common mechanisms.

In our experimental model, we observed that the NOP receptor selective agonist [Arg^{14},Lys^{15}]N/OFQ was able to mimic N/OFQ effects being approx 10-fold more potent than the natural peptide. These results are in line with previous data demonstrating that [Arg^{14},Lys^{15}]N/OFQ binds and fully activates the recombinant human NOP receptor with affinity/potency higher than N/OFQ while maintains high selectivity over classical opioid receptors (Okada *et al*., 2000). This pharmacological profile was later confirmed at native NOP receptors; in fact, [Arg^{14},Lys^{15}]N/OFQ behaved in various N/OFQ sensitive tissues as full agonist, displayed higher potency than N/OFQ (by three to more than 10 folds), and,

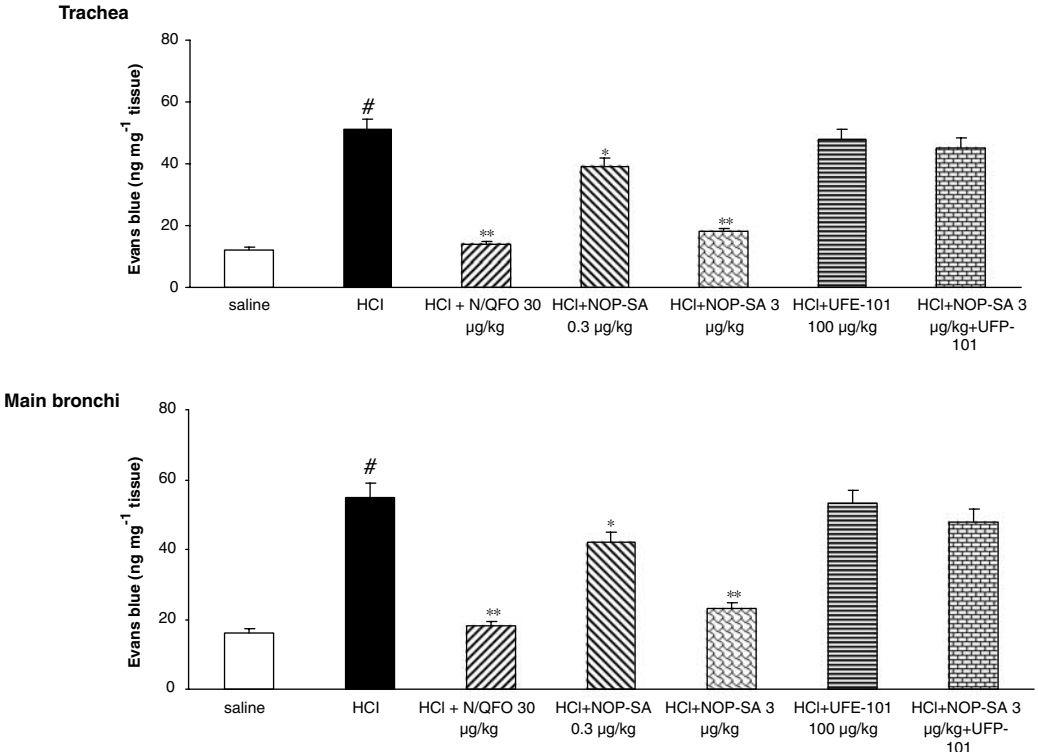

Figure 4 Comparison of the effects of NOP-SA (selective agonist of NOP receptor: [Arg^{14},Lys^{15}]N/OFQ), with respect to N/OFQ ($30\,\mu g\,kg^{-1}$) on the microvascular leakage induced by i.oe. HCl infusion (1 N) in anesthetized rabbits with or without NOP receptor (UFP-101) pretreatment. Rabbits were pretreated with atropine ($1\,mg\,kg^{-1}$), propranolol ($1\,mg\,kg^{-1}$) and phosphoramidon ($1\,mg\,kg^{-1}$). UFP-101 was administered 10 min before agonists. All values are expressed as mean ± s.e.m., $n=5$ per group. $^{\#}P<0.01$ vs saline; $^{*}P<0.05$ vs HCl; $^{**}P<0.01$ vs HCl.

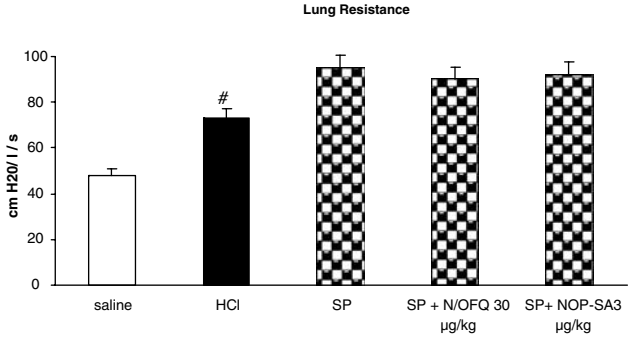

Figure 5 Effects of N/OFQ and NOP-SA (selective agonist of NOP receptor: [Arg^{14},Lys^{15}]N/OFQ) on SP-induced bronchoconstriction in anestethized rabbits pretreated with atropine ($1\,mg\,kg^{-1}$), propranolol ($1\,mg\,kg^{-1}$) and phosphoramidon ($1\,mg\,kg^{-1}$). SP ($0.3\,\mu g\,kg^{-1}$, i.v.) was injected 5 min before dye infusion. Data are expressed as mean ± s.e.m., $n=5$ per group; $^{\#}P<0.01$ vs saline.

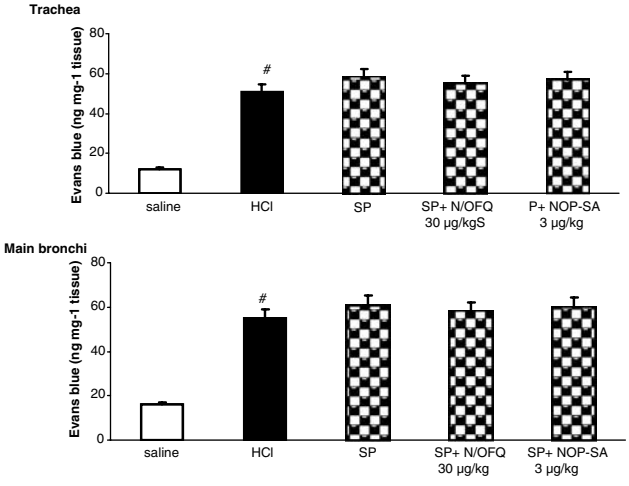

Figure 6 Effects of N/OFQ and NOP-SA (selective agonist of NOP receptor: [Arg^{14},Lys^{15}]N/OFQ) on SP-induced microvascular leakage of anestethized rabbits pretreated with atropine ($1\,mg\,kg^{-1}$), propranolol ($1\,mg\,kg^{-1}$) and phosphoramidon ($1\,mg\,kg^{-1}$). SP ($0.3\,\mu g\ kg^{-1}$, i.v.) was injected 5 min before dye infusion. Data are expressed as mean ± s.e.m., $n=5$ per group. $^{\#}P<0.01$ vs saline.

more importantly, its effects were antagonized by NOP antagonists ([Nphe^{1}]N/OFQ(1-13)NH$_2$ and J-113397) with pA$_2$ values similar to those obtained against N/OFQ (Rizzi *et al*., 2002).

More importantly, the actions of both NOP agonists were prevented by the peptide antagonist UFP-101 (Calò *et al*., 2000). The NOP selective antagonist properties of this molecule have been demonstrated in a variety of *in vitro* preparations ranging from cells expressing recombinant receptors (Calò *et al*., 2000; McDonald *et al*., 2003), to isolated tissues (Calò *et al*., 2000), and to brain preparations investigated with neurochemical (Marti *et al*., 2004; Mela *et al*., 2004) or electrophysiological (Gavioli *et al*., 2004; Marti *et al*., 2004) techniques. The pharmacological activity of UFP-101 has been also confirmed *in vivo* in rodents after central and

peripheral administration where the peptide antagonized N/OFQ actions on pain transmission (Calò *et al*., 2000), locomotor activity (Calò *et al*., 2000; Marti *et al*., 2004), neurotransmitter release (Koizumi *et al*., 2004; Marti *et al*., 2004) and cardiovascular parameters (Hashiba *et al*., 2003). Moreover, similar to other NOP selective antagonists (i.e. [Nphe^1]N/OFQ(1-13)NH_2 and J-113397) (Redrobe *et al*., 2002) UFP-101 produced antidepressant like effects in mice and rats (Gavioli *et al*., 2003; 2004). Interestingly enough, the antagonist/agonist dose ratio used in the present experiments for blocking N/OFQ effects is similar to that used in the above-mentioned studies (range 1–10) further suggesting that these drugs interact with the same functional sites, namely the NOP receptor.

As sensory nerves activation and endogenous tachykinins release seem to be involved in our experimental model of GER in the rabbit (Gallelli *et al*., 2003), our results suggest that NOP receptor activation inhibits sensory nerve activity. These results are in agreement with several lines of evidence indicating inhibitory actions of N/OFQ on sensory nerves in the guinea-pig, such as inhibition of the contraction of the isolated bronchus induced by EFS (Fischer *et al*., 1998), of capsaicin-induced bronchoconstriction of isolated lung (Corboz *et al*., 2000), of airway microvascular leakage induced by i.oe. HCl (Rouget *et al*., 2004), or in human isolated bronchi, inhibition of airway hyperresponsiveness induced by beta2-adrenoceptor agonists (Faisy *et al*., 2003). In addition, in this study, we also showed that N/OFQ and [Arg^{14},Lys^{15}]N/OFQ did not inhibit SP-induced bronchoconstriction and microvascular leakage in airways, suggesting that NOP agonists do not interact with postsynaptic neurokinin receptors, but exert their inhibitory effects on sensory nerves at presynaptic sites.

Since endogenous N/OFQ has been localized on nerve fibers within guinea-pig bronchus, and NOP receptor mRNA is expressed in jugular ganglion neuron cells, a direct inhibitory effect of N/OFQ on the release of tachykinins can be suggested (Fischer *et al*., 1996; 1998). Moreover, N/OFQ inhibits the micturition reflex in rats (Lecci *et al*., 2000) and in patients with neurogenic incontinence (Lazzeri *et al*., 2001; 2003) through its ability to activate inhibitory NOP receptor expressed on bladder C-fibers.

However, a vagally mediated autonomic pathway may also be involved in the experimental models described here, as Hamamoto *et al*. (1997) have shown that airway leakage induced by HCl was abolished in guinea-pigs by bilateral vagotomy. However, in our study, the effects of N/OFQ do not involve a cholinergic component of vagal activity since animals were pretreated by atropine. Our data cannot rule out a central action of N/OFQ. Indeed, several reports have documented the involvement of tachykinins (Mutoh *et al.,* 2000, Mazzone & Canning, 2002) in sensory nerve-mediated reflexes (Mazzone & Geraghty, 1999) related to the central control of cardiovascular and respiratory function by nucleus of the solitary tract (NTS). Since activation of NOP receptors, largely expressed in the NTS (Bunzow *et al*., 1994, Anton *et al*., 1996; Mao & Wang, 2000a), is able to reduce the NTS neuronal responsiveness to baroreceptor input (Mao & Wang, 2000a, b), we may thus suggest that N/OFQ is able to reduce sensory nerve excitability in this area (Dong *et al.,* 2002) and consequently to inhibit the bronchoconstriction and airway microvascular leakage evoked following esophageal acidification.

However, as N/OFQ is a long (17 aa) peptide and contains several positively charge residues, it is likely that the nerve endings and/or nerve connections between the esophagus and airways in the autonomic ganglia (Myers & Undem, 1993; Myers *et al*., 1996), where anatomical studies have demonstrated the presence of axon collaterals from visceral afferent fibers (gastrointestinal tract, bladder, airways) (Coleridge *et al*., 1989; Mawe, 1995; Canning *et al*., 1996; Myers *et al*., 1996), represent the site of action of N/OFQ.

Interestingly, Canning *et al*. (1998) have reported in an *in vitro* preparation of guinea-pig trachea and esophagus with their respective nerves that capsaicin elicited a concentration-dependent relaxation of the trachea only when the adjacent esophagus was intact. On the basis of the ability of tetrodotoxin to abolish the effect of capsaicin and of additional immunochemistry studies, it was concluded that the responses of the guinea-pig trachea induced by esophagus stimulation were mediated by nerve fibers from parasympathetic ganglia that are intrinsic to the adjacent esophagus (Canning *et al*., 1998). Similarly, in the present study, bronchoconstriction and airway microvascular leakage following esophageal acidification may occur as a result of the activation of the intermediate neurons including those from parasympathetic ganglia, by HCl stimulation of capsaicin-sensitive fibers in the esophagus.

Moreover, acid in the distal esophagus precipitates cough, and there is evidence for an esophageal–tracheobronchial cough reflex mechanism in patients with chronic cough associated with GER (Irwin *et al*., 1993). As sensory C-fibers also play a role in the generation of cough, this study may contribute to a better understanding of the pathophysiological mechanisms responsible for cough related to GER, thus improving pharmacotherapy.

In conclusion, our results suggest that bronchoconstriction and airway plasma extravasation induced by i.oe. HCl instillation are inhibited by NOP receptor activation, suggesting a possible use of N/OFQ and NOP receptor agonists for future investigations of the mechanisms involved in gastroesophageal-reflux induced bronchoconstriction and inflammation in asthmatic patients, and may represent a novel approach to the treatment of airway diseases relate to GER.

References

ADVENIER, C., D'AGOSTINO, B., CUI, Y.Y., EUTAMENE, H., ROUGET, C., BARDOU, M. & BUENO, L. (2002). Animal models for investigating gastroesophageal reflux disease-induced asthma. In: *Experimental and Clinical Pharmacology of Gastroesophageal Reflux-induced Asthma,* ed. Dal Negro, R.W., Geppetti, P. & Morice, A.H., pp. 51–54. Pisa: Pacini Editore.

ANTON, B., FEIN, J., TO, T., LI, X., SILBERSTEIN, L. & EVANS, C.J. (1996). Immunohistochemical localization of ORL-1 in the central nervous system of the rat. *J. Comp. Neurol.,* **368,** 229–251.

BERQUIST, W.E., RACHELEFSKY, G.S., KADDEN, M., SIEGEL, S.C., KATZ, R.M. & FONKALSRUD, E.W. (1981). Ament ME. Gastroesophageal reflux-associated recurrent pneumonia and chronic asthma in children. *Pediatrics,* **68,** 29–35.

BOLSER, D.C., MCLEOD, R.L., TULSHIAN, D.B. & HEY, J.A. (2001). Antitussive action of nociceptin in the cat. *Eur. J. Pharmacol.,* **430,** 107–111.

BUNZOW, W.J.R., SAEZ, C., MORTRUD, M., BOUVIER, C., WILLIAMS, J.T., LOW, M. & GRANDY, D.K. (1994). Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. *FEBS Lett.,* **347,** 284–288.

CALÒ, G., GUERRINI, R., RIZZI, A., SALVADORI, S. & REGOLI, D. (2000). Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br. J. Pharmacol.,* **129,** 1261–1283.

CALÒ, G., RIZZI, A., RIZZI, D., BIGONI, R., GUERRINI, R., MARZOLA, G., MARTI, M., MCDONALD, J., MORARI, M., LAMBERT, D.G., SALVADORI, S. & REGOLI, D. (2002). [Nphe1,Arg14,Lys15]nociceptin-NH2, a novel potent and selective antagonist of the nociceptin/orphanin FQ receptor. *Br. J. Pharmacol.,* **136,** 303–311.

CANNING, B.J., FISCHER, A. & UNDEM, B.J. (1998). Pharmacological analysis of the tachykinin receptors that mediate activation of nonadrenergic, noncholinergic relaxant nerves that innervate guinea pig trachealis. *J. Pharmacol. Exp. Ther.,* **284,** 370–377.

CANNING, B.J., UNDEM, B.J., KARAKOUSIS, P.C. & DEY, R.D. (1996). Effects of organotypic culture on parasympathetic innervation of guinea pig trachealis. *Am. J. Physiol.,* **271,** L698–L706.

COLERIDGE, H.M., COLERIDGE, J.C. & SCHULTZ, H.D. (1989). Afferent pathways involved in reflex regulation of airway smooth muscle. *Pharmacol. Ther.,* **42,** 1–63.

COLSON, D.J., CAMPBELL, C.A., WRIGHT, V.A. & WATSON, B.W. (1990). Predictive value of oesophageal pH variables in children with gastro-oesophageal reflux. *Gut,* **31,** 370–373.

CORBOZ, M.R., RIVELLI, M.A., EGAN, R.W., TULSHIAN, D., MATASI, J., FAWZI, A.B., BENBOW, L., SMITH-TORHAN, A., ZHANG, H. & HEY, J.A. (2000). Nociceptin inhibits capsaicin-induced bronchoconstriction in isolated guinea pig lung. *Eur. J. Pharmacol.,* **402,** 171–179.

COX, B.M., CHAVKIN, C., CHRISTIE, M.J., CIVELLI, O., EVANS, C., HAMON, M.D., HOELLT, V., KIEFFER, B., KITCHEN, I., MCKNIGHT, A.T., MEUNIER, J.C. & PORTOGHESE, P.S. (2000). Opioid receptors. In: *The IUPHAR Compendium of Receptor Characterization and Classification,* ed. Girdlestone, D., pp. 321–333. London: IUPHAR Media Ltd.

DAOUI, S., D'AGOSTINO, B., GALLELLI, L., ALT, X.E., ROSSI, F. & ADVENIER, C. (2002). Tachykinins and airway microvascular leakage induced by HCl intra-oesophageal instillation. *Eur. Respir. J.,* **20,** 268–273.

DONG, X.W., WILLIAMS, P.A., JIA, Y.P. & PRIESTLEY, T. (2002). Activation of spinal ORL-1 receptors prevents acute cutaneous neurogenic inflammation: role of nociceptin-induced suppression of primary afferent depolarization. *Pain,* **96,** 309–318.

FAISY, C., NALINE, E., ROUGET, C., GUEROT, E., FAGON, J.Y., CHINET, T. & ADVENIER, C. (2003). Nociceptin inhibits the *in vitro* fenoterol-induced neurosensitization of human bronchi. *Am. J. Respir. Crit. Care Med.,* **167,** A148.

FISCHER, A., FORSSMANN, W.G. & UNDEM, B.J. (1998). Nociceptin-induced inhibition of tachykinergic neurotransmission in guinea pig bronchus. *J. Pharmacol. Exp. Ther.,* **285,** 902–907.

FISCHER, A., MCGREGOR, G.P., SARIA, A., PHILIPPIN, B. & KUMMER, W. (1996). Induction of tachykinin gene and peptide expression in guinea pig no dose primary afferent neurons by allergic airway inflammation. *J. Clin. Invest.,* **98,** 2284–2291.

GALLELLI, L., D'AGOSTINO, B., MARROCCO, G., DE ROSA, G., FILIPPELLI, W., ROSSI, F. & ADVENIER, C. (2003). Role of tachykinins in the bronchoconstriction induced by HCl intraesophageal instillation in the rabbit. *Life Sci.,* **72,** 1135–1142.

GAVIOLI, E.C., MARZOLA, G., GUERRINI, R., BERTORELLI, R., ZUCCHINI, S., DE LIMA, T.C., RAE, G.A., SALVADORI, S., REGOLI, D. & CALO, G. (2003). Blockade of nociceptin/orphanin FQ-NOP receptor signalling produces antidepressant-like effects: pharmacological and genetic evidences from the mouse forced swimming test. *Eur. J. Neurosci.,* **17,** 1987–1990.

GAVIOLI, E.C., VAUGHAN, C.W., MARZOLA, G., GUERRINI, R., MITCHELL, V.A., ZUCCHINI, S., DE LIMA, T.C., RAE, G.A., SALVADORI, S., REGOLI, D. & CALO, G. (2004). Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. *Naunyn Schmiedebergs Arch. Pharmacol.,* **369,** 547–553.

GHAED, N. & STEIN, M.R. (1979). Assessment of a technique for scintigraphic monitoring of pulmonary aspiration of gastric contents in asthmatics with gastroesophageal reflux. *Ann. Allergy,* **42,** 306–308.

GUERRINI, R., CALO, G., RIZZI, A., BIANCHI, C., LAZARUS, L.H., SALVADORI, S., TEMUSSI, P.A. & REGOLI, D. (1997). Address and message sequences for the nociceptin receptor: a structure–activity study of nociceptin-(1-13)-peptide amide. *J. Med. Chem.,* **40,** 1789–1793.

HAMAMOTO, J., KOHROGI, H., KAWANO, O., IWAGOE, H., FUJII, K., HIRATA, N. & ANDO, M. (1997). Esophageal stimulation by hydrochloric acid causes neurogenic inflammation in the airways in guinea pigs. *J. Appl. Physiol.,* **82,** 738–745.

HARDING, S.M., GUZZO, M.R. & RICHTER, J.E. (2000). The prevalence of gastroesophageal reflux in asthma patients without reflux symptoms. *Am. J. Respir. Crit. Care Med.,* **162,** 34–39.

HASHIBA, E., HIROTA, K., KUDO, T., CALO, G., GUERRINI, R. & MATSUKI, A. (2003). Effects of nociceptin/orphanin FQ receptor ligands on blood pressure, heart rate, and plasma catecholamine concentrations in guinea pigs. *Naunyn Schmiedebergs Arch. Pharmacol.,* **367,** 342–347.

HERVÉ, P., DENJEAN, A., JIAN, R., SIMONNEAU, G. & DUROUX, P. (1986). Intraesophageal perfusion of acid increases the bronchomotor response to methacholine and to isocapnic hyperventilation in asthmatic subjects. *Am. Rev. Respir. Dis.,* **134,** 986–989.

IRWIN, R.S., FRENCH, C.L., CURLEY, F.J., ZAWACKI, J.K. & BENNETT, F.M. (1993). Chronic cough due to gastroesophageal reflux. Clinical, diagnostic, and pathogenetic aspects. *Chest,* **104,** 1511–1517.

ISHIKAWA, T., SEKIZAWA, T.I., SANT'AMBROGIO, F.B. & SANT'AMBROGIO, G. (1999). Larynx vs esophagus as reflexogenic sites for acid induced bronchoconstriction in dogs. *J. Appl. Physiol.,* **86,** 1226–1230.

JIA, Y., WANG, X., APONTE, S.I., RIVELLI, M.A., YANG, R., RIZZO, C.A., CORBOZ, M.R., PRIESTLEY, T. & HEY, J.A. (2002). Nociceptin/orphanin FQ inhibits capsaicin-induced guinea-pig airway contraction through an inward-rectifier potassium channel. *Br. J. Pharmacol.,* **135,** 764–770.

KJELLEN, G., BRUNDIN, A., TIBBLIG, L. & WRANNE, B. (1981). Oesophageal function in asthmatics. *Eur. J. Respir. Dis.,* **62,** 87–94.

KOIZUMI, M., MIDORIKAWA, N., TAKESHIMA, H. & MURPHY, N.P. (2004). Exogenous, but not endogenous nociceptin modulates mesolimbic dopamine release in mice. *J. Neurochem.,* **89,** 257–263.

LAZZERI, M., CALO, G., SPINELLI, M., GUERRINI, R., BENEFORTI, P., SANDRI, S., ZANOLLO, A., REGOLI, D. & TURINI, D. (2001). Urodynamic and clinical evidence of acute inhibitory effects of intravesical nociceptin/orphanin FQ on detrusor overactivity in humans: a pilot study. *J. Urol.,* **166,** 2237–2240.

LAZZERI, M., CALO, G., SPINELLI, M., GUERRINI, R., SALVADORI, S., BENEFORTI, P., SANDRI, S., REGOLI, D. & TURINI, D. (2003). Urodynamic effects of intravesical nociceptin/orphanin FQ in neurogenic detrusor overactivity: a randomized, placebo-controlled, double-blind study. *Urology,* **61,** 946–950.

LECCI, A., GIULIANI, S., MEINI, S. & MAGGI, C.A. (2000). Nociceptin and the micturition reflex. *Peptides,* **21,** 1007–1021.

MANSFIELD, L.E., HAMEISTER, H.H., SPAULDING, H.S., SMITH, N.J. & GLAB, N. (1981). The role of the vague nerve in airway narrowing caused by intraesophageal hydrochloric acid provocation and esophageal distention. *Ann. Allergy,* **47,** 431–434.

MANSFIELD, L.E. & STEIN, M.R. (1978). Gastroesophageal reflux and asthma: a possible reflex mechanism. *Ann. Allergy,* **41,** 224–226.

MAO, L. & WANG, J.Q. (2000a). Microinjection of nociceptin (Orphanin FQ) into nucleus tractus solitarii elevates blood pressure and heart rate in both anesthetized and conscious rats. *J. Pharmacol. Exp. Ther.,* **294,** 255–262.

MAO, L. & WANG, J.Q. (2000b). Pharmacological activation of nociceptin receptors in the nucleus tractus solitarius inhibits baroreceptor reflex in pentobarbital-anesthetized rats. *Neuroscience,* **101,** 435–440.

MARTI, M., MELA, F., VERONESI, C., GUERRINI, R., SALVADORI, S., FEDERICI, M., MERCURI, N.B., RIZZI, A., FRANCHI, G., BEANI, L., BIANCHI, C. & MORARI, M. (2004). Blockade of nociceptin/orphanin FQ receptor signaling in rat substantia nigra pars reticulata stimulates nigrostriatal dopaminergic transmission and motor behavior. *J. Neurosci.,* **24,** 6659–6666.

MAWE, G.M. (1995). Tachykinins as mediators of slow EPSPs in guinea-pig gall-bladder ganglia: involvement of neurokinin-3 receptors. *J. Physiol.,* **485,** 513–524.

MAZZONE, S.B. & CANNING, B.J. (2002). Synergistic interactions between airway afferent nerve subtypes mediating reflex bronchospasm in guinea pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **283,** R86–R98.

MAZZONE, S.B. & GERAGHTY, D.P. (1999). Respiratory action of capsaicin microinjected into the nucleus of the solitary tract: involvement of vanilloid and tachykinin receptors. *Br. J. Pharmacol.,* **127,** 473–481.

MCDONALD, J., CALO, G., GUERRINI, R. & LAMBERT, D.G. (2003). UFP-101, a high affinity antagonist for the nociceptin/orphanin FQ receptor: radioligand and GTPgamma(35)S binding studies. *Naunyn Schmiedebergs Arch. Pharmacol.,* **367,** 183–187.

MCLEOD, R.L., PARRA, L.E., MUTTER, J.C., ERICKSON, C.H., CAREY, G.J., TULSHIAN, D.B., FAWZI, A.B., SMITH-TORHAN, A., EGAN, R.W., CUSS, F.M. & HEY, J.A. (2001). Nociceptin inhibits cough in the guinea-pig by activation of ORL(1) receptors. *Br. J. Pharmacol.,* **132,** 1175–1178.

MELA, F., MARTI, M., ULAZZI, L., VACCARI, E., ZUCCHINI, S., TRAPELLA, C., SALVADORI, S., BEANI, L., BIANCHI, C. & MORARI, M. (2004). Pharmacological profile of nociceptin/orphanin FQ receptors regulating 5-hydroxytryptamine release in the mouse neocortex. *Eur. J. Neurosci.,* **19,** 1317–1324.

MEUNIER, J.C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.L., GUILLEMOT, J.C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, L. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature,* **377,** 532–535.

MOGIL, J.S. & PASTERNAK, G.W. (2001). The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol. Rev.,* **53,** 381–415.

MUTOH, T., BONHAM, A.C. & JOAD, J.P. (2000). Substance P in the nucleus of the solitary tract augments bronchopulmonary C fiber reflex output. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **279,** R1215–R1223.

MYERS, A.C. & UNDEM, B.J. (1993). Electrophysiological effects of tachykinins and capsaicin on guinea-pig bronchial parasympathetic ganglion neurones. *J. Physiol.,* **470,** 665–679.

MYERS, A., UNDEM, B. & KUMMER, W. (1996). Anatomical and electrophysiological comparison of the sensory innervation of bronchial and tracheal parasympathetic ganglion neurons. *J. Auton. Nerv. System,* **61,** 162–168.

OKADA, K., SUJAKU, T., CHUMAN, Y., NAKASHIMA, R., NOSE, T., COSTA, T., YAMADA, Y., YOKOYAMA, M., NAGAHISA, A. & SHIMOHIGASHI, Y. (2000). Highly potent nociceptin analog containing the Arg-Lys triple repeat. *Biochem. Biophys. Res. Commun.,* **278,** 493–498.

PATEL, H.J., GIEMBYCZ, M.A., SPICUZZA, L., BARNES, P.J. & BELVISI, M.G. (1997). Naloxone-insensitive inhibition of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by the novel opioid, nociceptin. *Br. J. Pharmacol.,* **120,** 735–736.

PEISER, C., UNDEM, B.J. & FISCHER, A. (2000). Nociceptin effects in the airways. *Peptides,* **21,** 995–998.

REDROBE, J.P., CALO, G., REGOLI, D. & QUIRION, R. (2002). Nociceptin receptor antagonists display antidepressant-like properties in the mouse forced swimming test. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **365,** 164–167.

REINSCHEID, R.K., NOTHACKER, H.P., BOURSON, A., ARDATI, A., HENNINGSEN, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA JR, F.J. & CIVELLI, O. (1995). Orphanin FQ: a neuropeptide that activates an opioid-like G protein-coupled receptor. *Science,* **270,** 792–794.

RICCIARDOLO, F.L. (2001). Mechanisms of citric acid-induced bronchoconstriction. *Am. J. Med.,* **111,** 18S–24S.

RIZZI, A., CALO, G., TREVISANI, M., TOGNETTO, M., FABBRI, L., MAPP, C., GUERRINI, R., SALVADORI, S., REGOLI, D. & GEPPETTI, P. (1999). Nociceptin receptor activation inhibits tachykinergic non adrenergic non cholinergic contraction of guinea pig isolated bronchus. *Life Sci.,* **64,** PL157–PL163.

RIZZI, A., RIZZI, D., MARZOLA, G., REGOLI, D., LARSEN, B.D., PETERSEN, J.S. & CALO, G. (2002). Pharmacological characterization of the novel nociceptin/orphanin FQ receptor ligand, ZP120: *in vitro* and *in vivo* studies in mice. *Br. J. Pharmacol.,* **137,** 369–374.

ROGERS, D.F., BELVISI, M.G., AURSUDKIJ, B., EVANS, T.W. & BARNES, P.J. (1988). Effects and interactions of sensory neuropeptides on airway microvascular leakage in guinea-pigs. *Br. J. Pharmacol.,* **95,** 1109–1116.

ROUGET, C., CUI, Y.Y., D'AGOSTINO, B., FAISY, C., NALINE, E., BARDOU, M. & ADVENIER, C. (2004). Nociceptin inhibits airway microvascular leakage induced by HCl intra-oesophageal instillation. *Br. J. Pharmacol.,* **141,** 1077–1083.

SCHLICKER, E. & MORARI, M. (2000). Nociceptin/orphanin FQ and neurotransmitter release in the central nervous system. *Peptides,* **21,** 1023–1029.

SHAH, S., PAGE, C.P. & SPINA, D. (1998). Nociceptin inhibits non-adrenergic non-cholinergic contraction in guinea-pig airway. *Br. J. Pharmacol.,* **125,** 510–516.

SPAULDING JR, H.S., MANSFIELD, L.E., STEIN, M.R., SELLNER, J.C. & GREMILLION, D.E. (1982). Further investigation of the association between gastroesophageal reflux and bronchoconstriction. *J. Allergy Clin. Immunol.,* **69,** 516–521.

THEODOROPOULOS, D.S., PECORARO, D.L. & EFSTRATIADIS, S.E. (2002). The association of gastroesophageal reflux disease with asthma and chronic cough in the adult. *Am. J. Respir. Med.,* **1,** 133–146.

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