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Nociceptin inhibits non-adrenergic non-cholinergic contraction in guinea-pig airway

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1 Electrical field stimulation (EFS) of guinea-pig isolated main bronchi induced a non-adrenergic non-cholinergic (NANC) contractile response. Nociceptin (0.01–1 μM) significantly inhibited the contractile response to EFS ($P<0.01$), but not to capsaicin ($P>0.05$).

2 The μ-, δ- and κ-opioid receptor antagonists, naloxone (0.3 μM), naltrindole (3 μM) and norbinaltorphimine (1 μM), respectively, did not significantly affect the inhibitory effect of nociceptin (0.03 μM; $P>0.05$).

3 The novel nociceptin antagonist, [Phe$^1\Psi$(CH_2-NH)Gly^2]nociceptin(1–13)NH_2 (0.03–1 μM); the σ ligands, carbetapentane (30 μM), 3-phenylpiperidine (30–100 μM) and (+)-cyclazocine (10–100 μM) significantly reversed the inhibitory effect of nociceptin (0.03 μM, $P<0.05$). In contrast, rimcazole, did not significantly reverse the inhibitory effect of nociceptin (0.03 μM) at any concentration tested ($P>0.05$).

4 EFS of guinea-pig bronchial preparations significantly increased SP-LI release above basal SP-LI ($P<0.05$). In the presence of nociceptin (1 μM), EFS induced a significant increase in SP-LI release above basal SP-LI release ($P<0.05$). Nociceptin caused a $59\pm11\%$ ($n=5$) inhibition of EFS-induced release of SP-LI.

5 Nociceptin reduces the release of sensory neuropeptides induced by EFS, but not capsaicin, from guinea-pig airways. These experiments provide further evidence for a role for nociceptin in regulating the release of sensory neuropeptides in response to EFS.

Keywords: Nociceptin; sensory neuropeptides; airways; Ψ-nociceptin; sigma ligands

Introduction

Recently, a novel opioid-like heptadecapeptide termed 'nociceptin' (Meunier *et al*., 1995) or 'orphanin FQ' (Reinscheid *et al*., 1995) has been isolated from brain tissue. Nociceptin is derived from a larger precursor termed prepronociceptin (PPNOC) whose rat, mouse and human genes have been isolated (Mollereau *et al*., 1996). The PPNOC gene is highly conserved in the three species and is strikingly similar to genes encoding the opioid precursors, i.e. preproenkephalin, preprodynorphin and preproopiomelanocortin. Nociceptin is the endogenous agonist for the 'orphan' receptor termed 'ORL_1' (Mollereau *et al*., 1994) or 'LC132' (Reinscheid *et al*., 1995). Like the μ, δ and κ opioid receptors, the ORI_1 receptor is G-protein-coupled and upon activation inhibits adenylate cyclase. *In vivo* studies in mice have demonstrated that nociceptin has central hyperalgesic properties (Meunier *et al*., 1995; Reinscheid *et al*., 1995), while at the spinal level in rats, it has antinociceptive properties *in vivo*, similar to opioids (Stanfa *et al*., 1996).

Excitatory non-adrenergic non-cholinergic (NANC) contractile responses have been reported in guinea-pig airway *in vitro* (Grundstrom *et al*., 1981) secondary to the release of neuropeptides such as substance P and neurokinin A. Morphine and opioid peptides have been shown to inhibit NANC contraction in guinea-pig airway (Frossard & Barnes, 1987; Kamikawa & Shimo, 1990), substance P release from rat trigeminal nucleus slices *in vitro* (Jessel & Iversen, 1977) and both capsaicin- and electrical-field stimulation (EFS)-induced NANC responses in guinea-pig perfused bronchial tubes (Lindstrom & Andersson, 1995). In recent studies, excitatory NANC contraction of guinea-pig renal pelvis (Giuliani & Maggi, 1996) and substance P release from rat trachea (Helyes *et al*., 1997) was inhibited by nociceptin.

We have investigated the effects of nociceptin on EFS- and capsaicin-induced contraction and the selectivity of various putative nociceptin antagonists, in guinea-pig isolated main bronchial preparations. Furthermore, we have investigated the effects of nociceptin on EFS-induced release of substance P-like immunoreactivity (SP-LI) from guinea-pig airway.

Methods

Tissue preparation

Male albino guinea-pigs (300–500 g) were killed by cervical dislocation and the lungs removed and placed in cold (4°C) Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 Main bronchial rings (2 mm) were suspended under 0.5 g tension, in 8 ml organ baths in Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 at 37°C, containing the cyclo-oxygenase inhibitor, indomethacin (5 μM) and the non-selective β-adrenoceptor antagonist, (−)-propranolol (1 μM). Changes in tension were measured, *via* a FTO3C transducer, and recorded using Maclab (version 3.3.8). Tissues were allowed to equilibrate for 40 min with changes in Krebs-Henseleit solution being made at 10 min intervals. Methacholine (0.3 and 10 μM) was added cumulatively to the bath to establish the sensitivity of the tissue and after the contractile response had reached plateau, the tissues were washed at 10 min intervals and allowed to equilibrate for a further 30 min.

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Electrical field stimulation studies

Guinea-pig isolated main bronchi were placed between two platinum electrodes and electrically stimulated (3 Hz, 20 s, 0.5 ms pulse width, 90 V) twice at 5 min intervals to determine the presence of any EFS-induced response. Tissues were then washed, incubated for 30 min in the presence of atropine (1 μM) and the neutral endopeptidase inhibitor, thiorphan (10 μM), and electrically stimulated (3 Hz, 20 s, 0.5 ms pulse width, 90 V). The resulting excitatory NANC contractile response returned to baseline after 30 min. Tissues were then electrically stimulated for a second time.

Contractile responses to EFS were obtained before and following 10 min incubation with nociceptin (0.01 – 1 μM). In further experiments, the effect of nociceptin (0.03 μM) on the contractile response to EFS was determined in the absence and presence of naloxone (0.3 μM), naltrindole (3 μM), nor-binaltorphimine (1 μM), [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin(1 – 13)NH$_2$ (ψ-nociceptin, 0.03 – 1 μM), carbetapentane (1 – 100 μM), 3-phenylpiperidine (3-PPP, 1 – 100 μM), (+)-cyclazocine (1 – 100 μM) and rimcazole (1 – 100 μM). One bronchial preparation from each animal was stimulated in the presence of vehicle (distilled water) to determine the effect of time-related fading on the contractile response to EFS. Tissues were incubated for 20 min in the presence of these drugs. In control experiments, the effect of 20 min incubation with the above mentioned drugs were examined for their effect on the excitatory NANC response. In other experiments, excitatory NANC contractile responses were obtained before and following 10 min incubation with [D-Ala^4,N-Me-Phe^4,Gly-ol^5]-enkephalin (DAMGO, 0.1 μM) in the absence or presence of the μ-opioid receptor antagonist, naloxone (0.3 μM; 20 min).

Data has been expressed as % inhibition of control which was calculated as the ratio of the difference between the first and the second excitatory NANC responses.

Substance P studies

In these experiments, the neutral endopeptidase inhibitor, thiorphan (10 μM) was added to the tissues after the initial response to methacholine had been obtained. Tissues were then incubated with vehicle or carbetapentane (100 μM), rimcazole (100 μM), 3-PPP (100 μM) and (+)-cyclazocine (100 μM) for 20 min prior to the addition of cumulative concentrations of substance P (0.0001 – 1 μM). After the contractile response has reached plateau, methacholine (10 μM) was added to the preparations. Data has been expressed as a percentage of the maximal response to methacholine (10 μM).

Capsaicin studies

In these experiments, the neutral endopeptidase inhibitor, thiorphan (10 μM) was added to the tissues after the initial response to methacholine had been obtained. Tissues were then incubated with vehicle or nociceptin (1 μM) for 10 min prior to the addition of cumulative concentrations of capsaicin (0.001 – 1 μM). After the contractile response has reached plateau, methacholine (10 μM) was added to the preparations. Data has been expressed as a percentage of the maximal response to methacholine (10 μM).

Substance P release studies

Male albino guinea-pigs (350 – 500 g) were killed by cervical dislocation and the trachea and lungs removed. The trachea, connective and parenchymal tissue were carefully excised until the isolated main bronchi and intrapulmonary bronchi remained. The bronchial tissue was placed in cold (4°C) Krebs-Henseleit solution and aerated with 95% O_2 and 5% CO_2. From each animal two bronchial preparations were obtained, each consisting of an isolated main bronchus and connected intrapulmonary bronchi.

The guinea-pig isolated bronchus and intrapulmonary bronchi were weighed, cut into 2 mm^3 pieces and placed on nylon gauze in a 1 ml perfusion bath. The tissue was superfused at a rate of 0.8 ml min^{-1} (Watson Marlow 503S2 peristaltic pump) with Krebs-Henseleit solution containing indomethacin (5 μM), (−)-propranolol (1 μM), thiorphan (10 μM) and 0.1% bovine serum albumin and aerated with 95% O_2 and 5% CO_2 at 37°C. The superfusion rate resulted in the tissues being constantly bathed in Krebs-Henseleit solution for stimulation purposes. Two platinum wire electrodes were then placed into the bath and connected to a SRI stimulator. Superfusate was collected at 3 min intervals after the tissues had equilibrated for 20 min.

The effect of nociceptin on substance P release was investigated by measuring EFS-induced release of substance P-like immunoreactivity (SP-LI) in the absence and presence of nociceptin (1 μM; 10 min preincubation) in two airway preparations obtained from each lung. Five fractions were collected at 3 min intervals in vials containing acetic acid (2 M). EFS (30 Hz, 0.5 ms pulse width, 100 V) was applied for 1 min immediately after the second fraction in the absence and presence of nociceptin (1 μM). All acidified fractions were freeze-dried under vacuum at −60°C (Edwards Freeze Dryer Modulyo) and then stored at −70°C. The basal release of SP-LI was determined from the mean of the first two fractions whilst EFS-induced release of SP-LI was determined from the remaining three fractions.

The amount of substance P-like immunoreactivity (SP-LI) in the collected fractions was determined using an enzyme linked immunoabsorbent assay (ELISA). Briefly, 96 well plates were coated prior to use with a mouse monoclonal anti-rabbit IgG antibody and stored at 4°C. The assay was carried out in a total volume of 150 μl in each well. The freeze-dried fractions from the experiments were reconstituted with 1 ml enzyme immunoassay (EIA) buffer and 50 μl was added to a well. Then 50 μl of substance P standards (7.8 – 1000 pg ml^{-1}) were added to other wells so as to construct a substance P standard curve. To complete the assay volume, 50 μl of acetylcholinesterase tracer and 50 μl of substance P antiserum were added to each standard and fraction well. The wells were incubated for 18 h at 4°C and then washed. Plates were then developed by the addition of 200 μl Ellman's reagent to each well. The wells were developed over 2 h in the dark and the absorbance was measured at 405 nm on a plate reader (Anthos Labtec HtIII). From the absorbance readings, the ratio of absorbance of substance P standard or fraction well (B) to that of the maximum binding (Bo) well was calculated and expressed as % B/Bo. For each unknown fraction, the % B/Bo value was identified on the standard curve and the corresponding substance P concentration was identified.

The sensitivity of this assay is 7.8 pg ml^{-1}. The EIA kit cross reacts to neurokinin A (2.7%), neurokinin B (0.04%) and eledoisin (12%). The amount of SP-LI from each fraction is expressed as pg g^{-1} tissue.

Analyses of results

Results from all experiments are expressed as mean ± s.e.mean. Analysis of variance (ANOVA) was used to determine

differences between control and drug treatment and differences between mean values were assessed with Student's non-paired t test. In the substance P studies, differences between the contractile potency ($pD_2 = -\log_{10}EC_{50}$) and the maximal response in the absence and presence of carbetapentane, rimcazole, 3-PPP and (+)-cyclazocine was compared with Student's non-paired t test. In the capsaicin studies, differences between the contractile potency ($pD_2 = -\log_{10}EC_{50}$) and the maximal response in the absence and presence of nociceptin was compared with Student's non-paired t test.

For the substance P release studies, data from control and nociceptin experiments were each analysed by repeated measures ANOVA to determine changes in SP-LI release following EFS. The difference between the mean basal SP-LI release and SP-LI release following EFS was determined in the absence and presence of nociceptin and compared with Student's non-paired t test. Differences between mean values were considered significant if $P<0.05$.

Drugs

Atropine, capsaicin, DAMGO, indomethacin, methacholine, (−)-propranolol, substance P, thiorphan, bovine serum albumin, sodium azide and ethylenediaminetetraacetic acid (EDTA) (Sigma Chemical Co., Dorset, U.K.). Nociceptin, Ψ-nociceptin (Tocris Cookson Ltd, Bristol. U.K.). Carbetapentane, (+)-cyclazocine, naloxone, naltrindole, nor-binaltorphimine, 3-PPP and rimcazole (Research Biochemical International, MA U.S.A.). Composition of Krebs-Henseleit solution (mM): NaCl 117.6, $NaHCO_3$ 25, Glucose 11.1, KH_2PO_4 1.03, $MgSO_4.7H_2O$ 0.57, KCl 5.4 and $CaCl_2$ 2.5. Krebs-Henseleit solution used in organ bath and substance P release experiments was prepared using distilled and ultra-pure water (18 MΩ), respectively. Stock concentrations of indomethacin (0.01 M), (−)-propranolol (0.01 M), thiorphan (0.01 M), substance P(0.001 M), capsaicin (0.01 M) and (+)-cyclazocine (0.01 M) were prepared in 0.5% Na_2CO_3, ultrapure water, 5% Na_2CO_3, 10% acetic acid, 100% ethanol and 0.1 M HCl respectively. Stock concentrations of methacholine (0.01 M) and atropine (0.01 M) were prepared in Krebs-Henseleit solution. Stock concentrations of nociceptin (0.001 M), Ψ-nociceptin (0.001 M) naloxone, (0.01 M), DAMGO (0.01 M), naltrindole (0.01 M), nor-binaltorphimine (0.001 M) carbetapentane (0.01 M), rimcazole (0.01 M), 3-PPP (0.01 M) were prepared in distilled water.

Substance P standard, substance P acetylcholinesterase tracer, substance P antiserum, mouse monoclonal anti-rabbit IgG antibody and Ellman's reagent (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). EIA buffer was composed of 100 ml of 0.1 M phosphate buffer at pH 7.4 containing sodium azide (10 mg), sodium chloride (2.34 g), EDTA (37 mg) and bovine serum albumin (0.1 g)

Results

Electrical field stimulation studies

EFS (3 Hz; 20 s; 0.5 ms pulse width; 90 V) of guinea-pig isolated main bronchi induced a contractile response, 45±3% ($n=25$) of the maximum response to methacholine (10 μM). In vehicle controls, no significant reduction in the contractile response to repeated EFS was observed (% methacholine E_{max}; 1st 40±6 vs 2nd 40±7, $n=20$, $P>0.05$).

Nociceptin significantly inhibited (% inhibition of excitatory NANC control) the contractile response to EFS (vehicle, −3±8, $n=12$ vs 0.01 μM, 38±7, $n=6$; 0.03 μM, 71±5, $n=6$; 0.1 μM, 78±4, $n=6$; 0.3 μM, 82±4, $n=6$; 1.0 μM, 97±7, $n=7$; $P<0.01$ cf. in absence of nociceptin (ANOVA) (Figure 1).

The μ-opioid receptor antagonist, naloxone (0.3 μM, 20 min preincubation), did not significantly reverse the inhibitory effect of nociceptin (0.03 μM) (% inhibition; control 65±8 vs naloxone 58±6, $n=5$, $P>0.05$, Figure 2a). However, naloxone significantly reversed the inhibitory effect of the μ-opioid agonist, DAMGO (0.1 μM, 10 min preincubation) on the EFS-induced contractile response (% inhibition; control 75±5 vs naloxone −11±17, $n=6$, $P<0.01$; Figure 2a).

The δ- and κ-opioid receptor antagonists, naltrindole (3 μM, 20 min preincubation) (% inhibition; control 65±8 vs naltrindole 73±6, $n=5$, $P>0.05$; Figure 2b) and norbinaltorphimine (1 μM, 20 min preincubation) (% inhibition; control 57±10 vs norbinaltorphimine 61±9, $n=5$, $P>0.05$; Figure 2b), respectively, did not antagonize the inhibitory effect of nociceptin (0.03 μM) on excitatory NANC contraction.

The putative nociceptin receptor antagonist, Ψ-nociceptin (0.03–1 μM, 20 min preincubation) significantly reversed the inhibitory effects of nociceptin (0.03 μM) on excitatory NANC contractile responses (% inhibition; absence, 61±5, $n=15$ cf in presence of Ψ-nociceptin, 0.03 μM, 16±8, $n=5$; 0.1 μM, 18±11, $n=5$; 1 μM, −5±5, $n=5$; $P<0.01$; Figure 3). However, Ψ-nociceptin alone, caused significant inhibition of the contractile response to EFS (% inhibition; vehicle −3±5, vs 0.03 μM, 23±9, $n=5$; 0.1 μM, 33±7, $n=5$; 1 μM, 32±9, $n=5$, $P<0.01$ cf. to vehicle).

The σ ligand, carbetapentane (30 μM) significantly reversed the inhibitory effect of nociceptin (0.03 μM) on excitatory NANC contractile responses (% inhibition; absence, 59±5, $n=16$ cf. in presence of carbetapentane 21±6, $n=5$, $P<0.05$; Figure 4a). Similarly, 3-PPP (30–100 μM) (absence, 63±5, $n=15$ cf. in presence of 3-PPP, 30 μM, 17±5, $n=5$; 100 μM, 15±6, $n=5$; $P<0.01$; Figure 4b) and (+)-cyclazocine, (10–100 μM) (absence, 70±5, $n=14$ cf. in presence of (+)-cyclazocine, 10 μM, 41±9, $n=5$; 30 μM, 32±7, $n=5$; 100 μM, 9±6, $n=5$, $P<0.05$; Figure 5a), significantly reversed the inhibitory effect of nociceptin (0.03 μM) on excitatory NANC contractile responses. In contrast, rimcazole, did not significantly reverse the inhibitory effect of nociceptin (0.03 μM) at all concentrations tested ($P>0.05$; Figure 5b). High concentrations of carbetapentane (100 μM) and rimcazole (100 μM) alone, significantly inhibited the contractile response

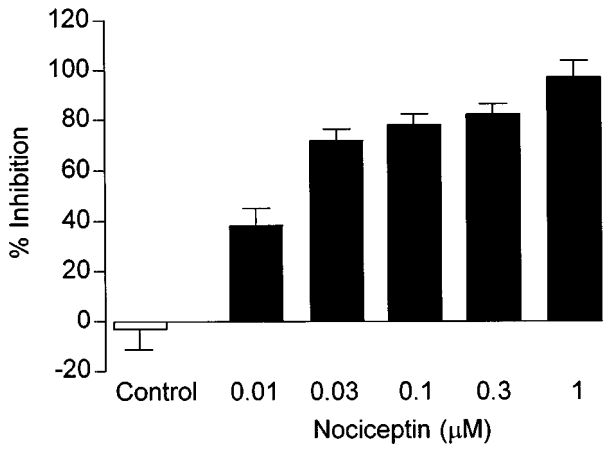

Figure 1 Concentration-dependent inhibition by nociceptin (closed columns, $n=6–7$) of the excitatory NANC contractile response to EFS in guinea-pig isolated bronchi. Control values (open columns, $n=12$). Each value represents the mean±s.e.mean.

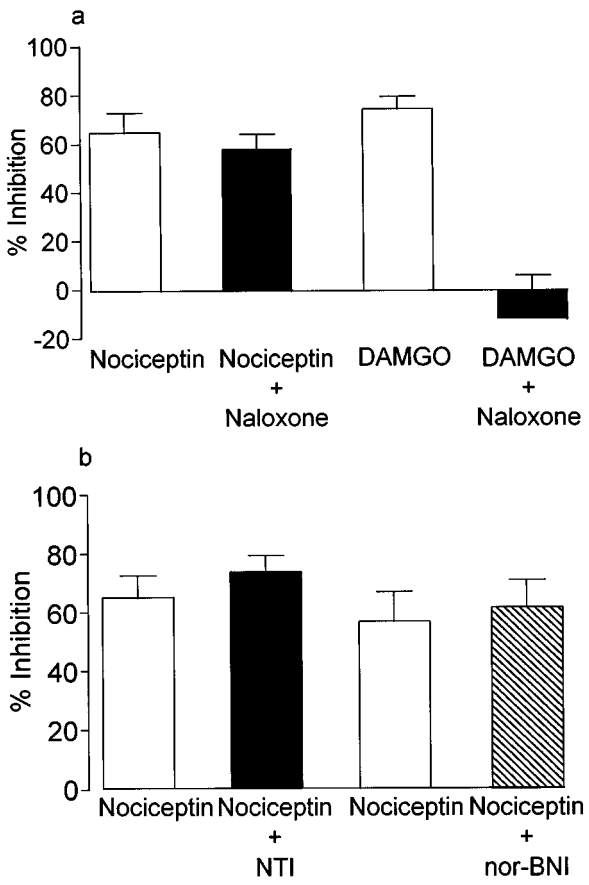

Figure 2 Effects of, (a) naloxone (0.3 μM) on nociceptin (0.03 μM)- and DAMGO (0.1 μM)-induced, and (b) naltrindole (NTI; 3 μM) and nor-binaltorphimine (nor-BNI; 1 μM) on nociceptin-induced inhibition of the excitatory NANC contractile response to EFS in guinea-pig isolated bronchi. Absence (open columns) and presence (closed or cross-hatched columns) of opioid receptor antagonists Each value is the mean $\pm$ s.e. mean of 5–6 experiments.

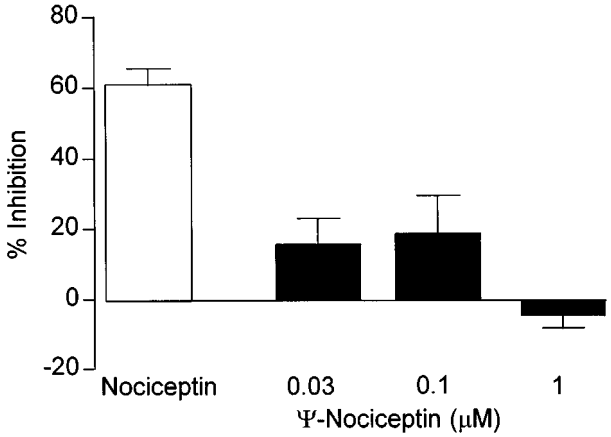

Figure 3 Inhibition by nociceptin (0.03 μM) of the EFS-induced excitatory NANC contractile response in guinea-pig isolated bronchi in the absence (open columns) or presence (closed columns) of the putative nociceptin antagonist, Ψ-nociceptin. Each value is the mean $\pm$ s.e.mean of 5–15 experiments.

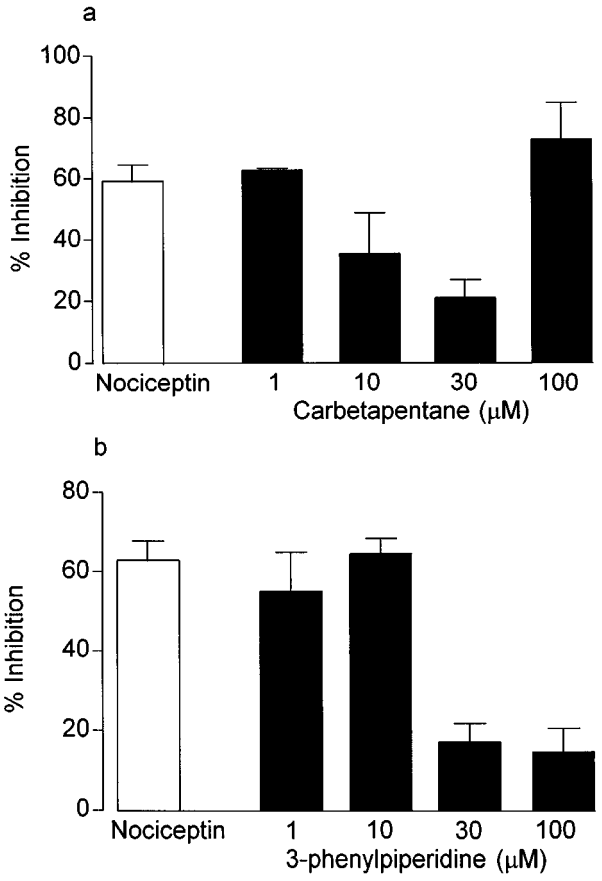

Figure 4 Inhibition by nociceptin (0.03 μM) of the EFS-induced excitatory NANC contractile response in guinea-pig isolated bronchi in the absence (open columns) or presence (closed columns) of the σ ligands, (a) carbetapentane, and (b) 3-phenylpiperidine. Each value is the mean $\pm$ s.e.mean of 5–16 experiments.

to EFS ($P<0.01$; Table 1). However, 3-PPP and (+)-cyclazocine alone, were without effect ($P>0.05$; Table 1).

Substance P studies

Neither carbetapentane, 3-PPP nor (+)-cyclazocine (100 μM) altered the contractile potency (pD_2) or the maximum contractile response to substance P. In contrast, rimcazole (100 μM), significantly reduced the contractile potency and the maximum contractile response to substance P (Table 2).

Capsaicin studies

Capsaicin induced a concentration-dependent contractile response in guinea-pig isolated bronchi with a potency (pD_2) of 7.1 ± 0.17 ($n=9$) that was not reduced in the presence of nociceptin (1 μM; 6.9 ± 0.14, $n=9$, $P>0.05$; Figure 6). The maximal contractile responses to capsaicin (% methacholine E_{max}) was not significantly altered in the presence of nociceptin (1 μM; absence, 78 ± 6, $n=9$ vs presence, 73 ± 7, $n=9$, $P>0.05$).

Substance P release studies

Electrical field stimulation (3 Hz, 1 min, 0.5 ms pulse width, 100 V) of guinea-pig bronchial preparations significantly increased SP-LI (pg g^{-1} tissue) release at 9 min above basal SP-LI (mean basal 62 ± 23 vs EFS 455 ± 28, $n=5$, $P<0.01$). In the presence of nociceptin (1 μM), EFS induced a significant increase in SP-LI release at 9 min above basal SP-LI (mean basal 86 ± 26 vs EFS 238 ± 53, $n=5$, $P<0.05$; Figure 7).

Table1 Effect of various σ ligands alone, on the excitatory NANC contractile response to EFS in guinea-pig bronchus

	Vehicle	*1 μM*	*10 μM*	*30 μM*	*100 μM*
Carbetapentane	−2±7	11±23	−18±13	−59±14**	64±7**
Rimcazole	−1±7	34±16	19±14	36±16	72±10**
3-PPP	−3±5	4±2	7±9	7±6	−12±15
(+)cyclazocine	3±6	2±7	−6±9	−11±11	−18±7

% inhibition values are shown as mean±s.e.mean of 5–16 experiments, negative values indicate augmentation of the excitatory NANC response, $^{**}P<0.01$ cf. with vehicle.

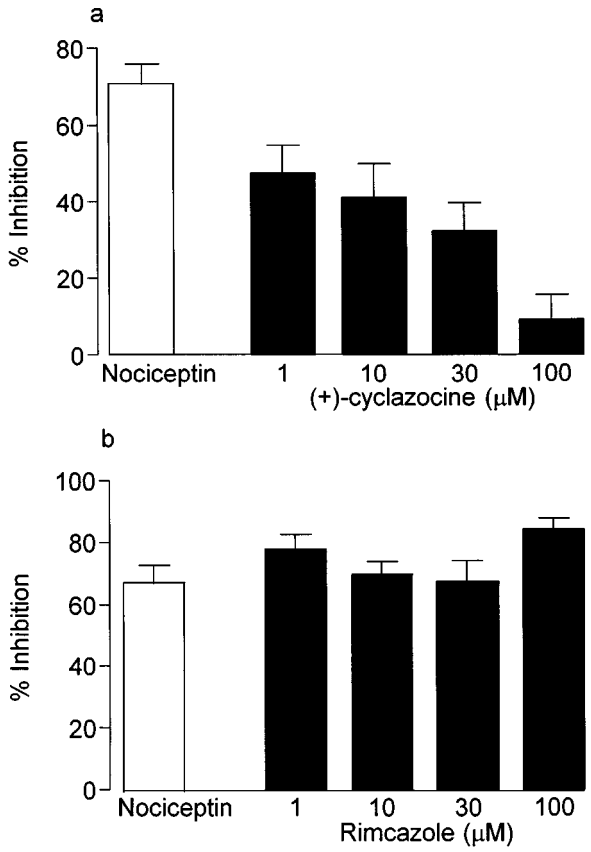

Figure 5 Inhibition by nociceptin (0.03 μM) of the EFS-induced excitatory NANC contractile response in guinea-pig isolated bronchi in the absence (open columns) or presence (closed columns) of the σ ligands, (a) (+)-cyclazocine, and (b) rimcazole. Each value is the mean±s.e.mean of 5–15 experiments.

Table 2 Contractile potency (pD_2) and maximum contractile response (% methacholine E_{max}) values for substance P in the absence and presence of various σ ligands

	pD_2	% E_{max}	n
Control	7.6±0.5	85±6	6
Carbetapentane (100 μM)	7.4±0.1	96±1	6
Control	7.5±0.1	94±2	5
Rimcazole (100 μM)	6.8±0.1*	71±5*	5
Control	7.9±0.1	96±3	5
3-PPP (100 μM)	7.7±0.3	92±5	5
Control	7.5±0.2	92±2	5
(+)-Cyclazacone (100 μM)	7.3±0.2	87±3	5

Values are shown as mean±s.e.mean, *n* represents the number of preparations, $^{*}P<0.05$ cf. with control.

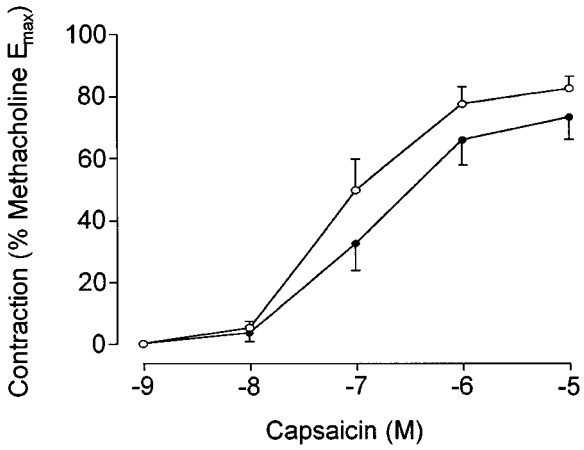

Figure 6 Concentration-response curves to capsaicin in the absence (○) or presence (●) of nociceptin (1 μM). Each value is the mean±s.e.mean of 9 experiments.

Nociceptin caused a 59±11% inhibition ($n=5$) of EFS-induced release of SP-LI ($P<0.05$).

Discussion

We have demonstrated that the novel opioid-like peptide, nociceptin, inhibited excitatory NANC contractile responses induced by EFS but not capsaicin in guinea-pig isolated bronchus. This inhibitory effect, was independent upon the activation of either μ-, δ- or κ-opioid receptors as seen by the inability of the respective opioid receptor antagonists to reverse the inhibitory effect of nociceptin. The putative nociceptin receptor antagonist, Ψ-nociceptin, was able to reverse the inhibitory effects of nociceptin, although it also inhibited the excitatory NANC contractile response. The σ ligands, carbetapentane, 3-PPP and (+)-cyclazocine but not rimcazole, antagonized the inhibitory effect of nociceptin albeit at high concentrations. We also demonstrated that nociceptin inhibited the electrically-induced release of substance P-like immunoreactivity from guinea-pig isolated bronchus.

Nociceptin is a recently discovered opioid-like peptide isolated from brain tissue which appears to act on specific orphan (ORL1) receptors (Mollereau *et al*., 1994; Meunier *et al*., 1995; Reinscheid *et al*., 1995) and causes the activation of potassium channels (Connor *et al*., 1996; Kobayashi *et al*., 1997; Vaughan *et al*., 1997), inhibition of cyclic AMP accumulation in neuroblastoma cells (Ma *et al*., 1997) and inhibition of N-type calcium currents (Buritova *et al*., 1996). Furthermore, nociceptin inhibits acetylcholine release from guinea-pig isolated trachea (Patel *et al*., 1997), relaxes carotid and femoral arteries (Gumusel *et al*., 1997), inhibits glutamate

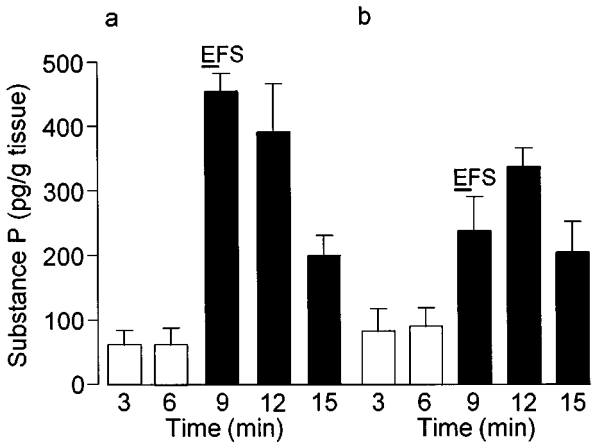

Figure 7 Release of substance P-like immunoreactivity (SP-LI) from guinea-pig bronchial preparations in response to EFS (bar, 1 min) in (a) absence and (b) presence of nociceptin (1 μM). Basal release of SPLI (open columns) and EFS-induced release of SP-LI (closed columns). Each value is the mean $\pm$ s.e.mean of 5 experiments.

release from rat cerebrocortical slices (Nicol *et al*., 1996) and dorsal ventral root (Faber *et al*., 1996). Nociceptin has also been demonstrated to inhibit dopamine release from nucleus accumbens (Murphy *et al*., 1996), excitatory NANC contractile responses in guinea-pig isolated renal pelvis (Giuliani & Maggi, 1996) and substance P release from rat trachea (Helyes *et al*., 1997). Nociceptin failed to alter the mechanosensitivity of jugular afferent C-fibres in guinea-pig airway (Undem *et al*., 1997). Consistent with these studies, we have demonstrated that nociceptin inhibited excitatory NANC contractile response in a concentration-dependent manner, an effect attributable to a pre-synaptic mechanism of action as confirmed from our release studies. Thus, nociceptin inhibited the release of substance P-like immunoreactivity following electrical field stimulation of guinea-pig airways. The μ-opioid receptor antagonist, naloxone, at a concentration that antagonized the inhibitory effect of the μ-opioid receptor agonist, DAMGO (Bartho *et al*., 1987; Frossard & Barnes, 1987), was ineffective against nociceptin in this study. Moreover, the δ- and κ-opioid receptor antagonists, naltrindole and nor-binaltorphimine respectively, were without effect upon the excitatory NANC contractile response. These data are consistent with the finding that the inhibitory effect of nociceptin on excitatory NANC responses in guinea-pig renal pelvis was not dependent upon the activation of μ, δ- and κ-opioid receptors (Giuliani & Maggi, 1996).

Recently, the σ ligands, carbetapentane and rimcazole have been reported to act as antagonists of the ORL_1 receptor in *Xenopus* oocytes (Kobayashi *et al*., 1997). Sigma (σ) ligands are a diverse group of compounds comprised of guanidines, benzomorphans, butyrophenones, peptides and steroids (Walker *et al*., 1990). σ ligands have been shown to have psychotomimetic, antipsychotic, neuroprotective, antitussive and antiepileptic properties (Walker *et al*., 1990). We demonstrated that only high concentrations of carbetapentane, 3-PPP and (+)-cyclazocine reversed the inhibitory effect of nociceptin on the excitatory NANC contractile response, while rimcazole totally lacked any antagonistic action. In contrast, both carbetapentane and rimcazole have been reported to antagonize the inhibitory effect of nociceptin on inward potassium currents in *Xenopus* oocytes with IC50 values of 9 and 12 μM respectively. However, 3-PPP and (+)-cyclazocine did not antagonize the inhibitory effect of nociceptin in *Xenopus* oocytes (Kobayashi *et al*., 1997). Furthermore, in our study, high concentrations of rimcazole was found to inhibit the EFS-induced excitatory NANC contractile response and the substance P-induced contractile response, suggesting that it behaves as a functional antagonist in this preparation. High concentrations of carbetapentane directly modulated the excitatory NANC response, the mechanism of which remains to be established, but is unlikely to be due to a postjunctional mechanism of action.

Recently, a novel nociceptin antagonist, Ψ-nociceptin, has been described (Guerrini *et al*., 1998). Ψ-nociceptin was shown to reverse the inhibitory effects of nociceptin on EFS-induced contractions in guinea-pig ileum and mouse vas deferens with pA_2 values of 7.02 and 6.75, respectively. While we demonstrated that Ψ-nociceptin reversed the inhibitory action of nociceptin, we found that Ψ-nociceptin (0.03 – 1 μM) alone, inhibited excitatory NANC contraction suggesting it behaves as a partial agonist in guinea-pig bronchus.

We have also investigated the effect of nociceptin on contractile responses elicited by capsaicin which is known to stimulate the opening of a non-selective ion channel on sensory C-fibres (Maggi, 1995). Nociceptin failed to significantly inhibit the contractile response to capsaicin and is consistent with a range of literature reports documenting the inability of galanin (Giuliani *et al*., 1989a), neuropeptide Y (Giuliani *et al*., 1989b), μ-opioids (Bartho *et al*., 1987), phosphodiesterase type 4 isoenzyme inhibitors (Undem *et al*., 1994; Spina *et al*., 1995) and the phosphatase 1 and 2A inhibitor okadaic acid (Harrison *et al*., 1997) to inhibit contractions induced by capsaicin. This suggests that while opioid receptors and the ORL_1 receptor have highly similar gene sequences (Reinscheid *et al*., 1995), both may have little effect on the direct release of sensory neuropeptides from peripheral sensory nerves following activation of the capsaicin receptor. Release of sensory neuropeptides following nerve depolarization however, is mediated via opening of N-type Ca^{2+} channels both of which are regulated by opioids (Seward *et al*., 1991) and nociceptin (Connor *et al*., 1996).

In conclusion, our study has demonstrated that nociceptin inhibits sensory neuropeptide release induced by EFS from guinea-pig airway, via a non-opioid-mediated mechanism. The inhibitory effect of nociceptin is reversed by the novel nociceptin antagonist, Ψ-nociceptin.

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