Br. J. Pharmacol. (1988), **95**, 413–418

Opioid modulation of non-cholinergic neural bronchoconstriction in guinea-pig *in vivo*

M.G. Belvisi, K.F. Chung, D.M. Jackson & [1]P.J. Barnes

Department of Thoracic Medicine, Cardiothoracic Institute, Brompton Hospital, London SW3 6HP

1 Opioid receptors have been demonstrated on sensory fibres in the vagus nerve. Non-cholinergic (NC) neural bronchoconstriction in guinea-pig is due to release of neuropeptides from sensory nerve endings. We have therefore studied the effect of opioids on this NC bronchoconstriction in the anaesthetized guinea-pig.

2 Bilateral vagal stimulation (5 V, 5 ms, 10 Hz) caused reproducible bronchoconstriction in guinea-pigs which was reduced by atropine ($1\,mg\,kg^{-1}$), but the NC component was unaffected by hexamethonium ($10\,mg\,kg^{-1}$).

3 NC bronchoconstriction was reduced by morphine in a dose-dependent manner ($ED_{50} = 132\,\mu g\,kg^{-1}$ with a maximal inhibition of $79 \pm 2.1\%$ at $1\,mg\,kg^{-1}$). Yohimbine ($0.5\,mg\,kg^{-1}$) did not alter the inhibitory effect of morphine ($1\,mg\,kg^{-1}$).

4 The inhibitory effect of morphine was completely reversed by naloxone ($1\,mg\,kg^{-1}$) which had no effect on NC bronchoconstriction. Propranolol ($1\,mg\,kg^{-1}$) significantly increased the NC bronchoconstrictor response but did not significantly alter the inhibition by morphine.

5 The selective μ-opioid receptor agonist Tyr-(D-Ala)-Gly-(N-Me-Phe)-Glyol (DAGOL) was significantly more potent than morphine with an ED_{50} of $5.4\,\mu g\,kg^{-1}$ and complete inhibition at $100\,\mu g\,kg^{-1}$. The δ-agonist Tyr-(D-Pen)-Gly-Phe-(D-Pen) (DPDPE) was less potent than DAGOL with an ED_{50} of $28\,\mu g\,kg^{-1}$ and a maximal inhibition of only $50 \pm 5\%$ at $100\,\mu g\,kg^{-1}$. The κ-receptor agonist, U-50,488H had no inhibitory effect on the NC bronchoconstrictor response.

6 The bronchoconstrictor responses to exogenous substance P ($25\,\mu g\,kg^{-1}$) or acetylcholine ($25\,\mu g\,kg^{-1}$) were unaffected by morphine ($500\,\mu g\,kg^{-1}$).

7 We conclude that opioids inhibit the NC bronchoconstrictor response to vagal stimulation via an action on μ-opioid receptors localized to sensory nerve endings in the airway.

Introduction

Opioid receptors have been localized to sensory fibres of the vagus nerves (Atweh *et al.*, 1978; Young *et al.*, 1980). Selective destruction of unmyelinated afferent nerves by capsaicin leads to a reduction in opioid binding sites (Nagy *et al.*, 1980; Laduron, 1984) suggesting that opioid receptors are present on a capsaicin-sensitive population of sensory nerves. These nerves contain neuropeptides including substance P (SP) (Lundberg *et al.*, 1983a). Opioids have been shown to inhibit the release of SP from the rat trigeminal nucleus *in vitro* (Jessell & Iversen, 1977) and neurogenic vasodilatation and plasma extravasation in rat hind paw (Bartho & Szolcsanyi, 1981; Lembeck *et al.*, 1982; Smith & Buchan, 1984; Lembeck & Donnerer, 1985).

[1] Author for correspondence.

Non-cholinergic bronchoconstriction caused by vagal stimulation *in vivo* and by electrical field stimulation *in vitro* may be due to the release of neuropeptides such as SP and neurokinins from sensory nerves (Lundberg *et al.*, 1983b; Andersson & Grundstrom, 1983). Opioids inhibit the non-cholinergic (NC) constrictor response in guinea-pig bronchi *in vitro* in a dose-dependent manner (Frossard & Barnes, 1987). We have now investigated whether opioids similarly inhibit NC bronchoconstriction *in vivo* in the same species. We have examined the effects of morphine, and DAGOL (Tyr-(D-Ala)-Gly-(N-Me-Phe)-Gly-ol), DPDPE (Tyr-(D-Pen)-Gly-Phe-(D-Pen)) and U-50,488H (*trans*-3, 4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl) -benzeneacetamine), selective agonists of μ-, δ- and κ-opioid receptors, respectively.

Some of these results were presented at the September 1987 meeting of the British Pharmacological Society (Belvisi *et al.*, 1987).

Methods

Male Dunkin-Hartley outbred guinea-pigs (Charles River U.K. Ltd., Kent) 300–600 g body weight were housed in a temperature-controlled (21°C) room with food and water freely available. They were anaesthetized with an intraperitoneal injection of urethane (8 ml kg^{-1} of a 25% solution w/v in saline) and placed on a heated blanket (Homeothermic system, Harvard Apparatus Ltd., Edenbridge, Kent, England) which maintained body temperature at 37°C. The left jugular vein was cannulated for injection of drugs. The left carotid artery was cannulated for monitoring blood pressure via an indwelling Portex cannula filled with heparin-saline (10 u ml^{-1}) linked to a pressure transducer (Druck blood pressure transducer, Druck Ltd., Groby, Leicestershire) connected to a recorder (Lectromed Multitrace 2, Ormed Ltd., Welwyn Garden City, Hertfordshire). The trachea was cannulated and the animal ventilated with a small animal constant volume respiration pump (Bioscience U.K., Sheerness, Kent), operating at 60 strokes min^{-1} of 1 ml laboratory air per 100 g body weight. Overflow pressure was measured by a modification of the Konzett-Rossler procedure (Konzett & Rossler, 1940) with a differential pressure transducer (Farnell Electronic Components Ltd., Leeds). Both cervical vagus nerves were carefully dissected free, sectioned (to avoid stimulating the CNS) and their peripheral ends placed on platinum electrodes. A Fenton Lewis Double-channel Stimulator MK IV (Wallington Instruments, Purley, Surrey) was used to stimulate the nerves electrically using pulses of 5 ms at 10 Hz and 5 V for 30 s.

Experimental protocol

The vagi were stimulated and the effect on overflow and blood pressure observed. Atropine (1 mg kg^{-1}) was administered intravenously (i.v.) and the vagi were stimulated again after 30 min to give a NC bronchoconstrictor response. Hexamethonium (10 mg kg^{-1}) and propranolol (1 mg kg^{-1}) were also used in some experiments. Vagal stimulation was repeated again 10 min later or when the overflow pressure had returned to its baseline value. After two identical responses had been elicited the effect of opioids was investigated. Morphine (100–1000 $\mu g\,kg^{-1}$), DAGOL (1–100 $\mu g\,kg^{-1}$), DPDPE (10–100 $\mu g\,kg^{-1}$) or U-50,488H (1000 $\mu g\,kg^{-1}$), administered i.v. 2 min before vagal stimulation, were studied using only a single dose in each animal. The action of morphine (1 mg kg^{-1}) in the presence of the α-adrenoceptor antagonist yohimbine (0.5 mg kg^{-1}) was examined. The time course of action of morphine (1 mg kg^{-1}) was determined.

The effect of morphine (500 $\mu g\,kg^{-1}$) on bronchoconstriction evoked by either exogenous SP (25 $\mu g\,kg^{-1}$), exogenous acetylcholine (ACh, 25 $\mu g\,kg^{-1}$) or vagal stimulation without atropine were also studied in separate experiments. After achieving inhibition with morphine, naloxone (1 mg kg^{-1}) was injected and 10 min later the vagi were stimulated to see if reversal of the opioid effect was possible. Naloxone was also injected alone in other experiments to determine whether endogenous opioids had an inhibitory effect on NC bronchoconstriction at these stimulation parameters.

Drugs

Drugs and chemicals were obtained from the following sources: SP, urethane, acetylcholine chloride, yohimbine hydrochloride, hexamethonium chloride (Sigma Chemical Co., Poole, Dorset), atropine sulphate BP (Phoenix Pharmaceuticals Ltd., Oxford), propranolol hydrochloride (Imperial Chemical Industries, Cheshire), naloxone hydrochloride (Du Pont U.K., Hertfordshire), morphine hydrochloride (May and Baker, Dagenham, Essex), DAGOL, DPDPE (Bachem Feinchemikalien AG, Bubendorf, Switzerland), U-50,488H (The Upjohn Company, Kalamazoo, U.S.A), heparin injection BP (CP Pharmaceuticals Ltd., Wrexham). All drugs were soluble in saline.

Statistical analyses

Mean arterial blood pressure (BP) was calculated from recorded traces as: diastolic pressure +0.33 (systolic pressure − diastolic pressure). Results are presented as means ± s.e.mean. Differences between means were analysed by Student's t test for paired data (two-tailed). A probability level of $P < 0.05$ was considered statistically significant. The dose of drug required to produce 50% of the maximal inhibition of the NC bronchoconstrictor response evoked by vagal stimulation (ED_{50}) was read directly from curves, constructed using % maximal responses as a function of the dose of opioid.

Results

Effect of vagal stimulation

Vagal stimulation produced a significant bronchoconstrictor response that was reproducible within

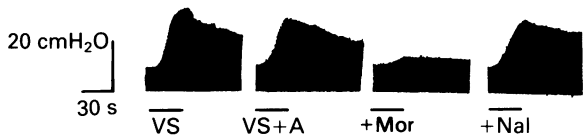

Figure 1 Tracing of tracheal pressure in response to vagal stimulation (VS), at 10 Hz, 5 V, 5 ms for 30 s, before and after atropine (A, 1 mg kg^{-1}) in a single guinea-pig. The effect of morphine (Mor, 500 μg kg^{-1}) on the non-cholinergic bronchoconstrictor response is shown. The reversal of the morphine effect by naloxone (Nal, 1 mg kg^{-1}), administered after morphine, is also shown.

any given animal (maximal increase in airway pressure = 23.6 ± 1.9 cmH_2O, $n = 19$) which was reduced after atropine (1 mg kg^{-1}) to 12.6 ± 1.4 cmH_2O ($n = 19$) (Figure 1). Hexamethonium (10 mg kg^{-1}) had no significant effect on the NC response when injected 2 min before stimulation ($n = 4$). Propranolol (1 mg kg^{-1}) significantly ($P < 0.001$) increased the NC bronchoconstrictor response to 27.6 ± 2.7 cmH_2O ($n = 16$) when injected together with atropine 30 min before stimulation.

Effect of morphine on the NC bronchoconstrictor response

Morphine, when injected 2 min before stimulation, caused a dose-dependent decrease in the NC excitatory component evoked by vagal stimulation; at a dose of 100 μg kg^{-1} the inhibition was 31.4 ± 5.9% ($n = 5$) ($P < 0.05$) and at 500 μg kg^{-1} 76.2 ± 5.7% ($n = 6$) ($P < 0.01$) (Figure 2). The inhibitory effect of morphine (1 mg kg^{-1}) was present for at least 30 min but had reversed by 60 min (Figure 3). Morphine inhibited the bronchoconstrictor response evoked by vagal stimulation in the absence of atropine (77 ± 6.8% inhibition with morphine 500 μg kg^{-1}, $n = 5$) ($P < 0.001$) and the inhibitory effect was similar to that seen in the presence of atropine. Naloxone (1 mg kg^{-1}) completely reversed the inhibitory effect of morphine (500 μg kg^{-1}) ($n = 5$) when injected 10 min before stimulation but there was still considerable inhibition of the NC bronchoconstrictor response (62.6 ± 8.1%) (Figure 1). Propranolol (1 mg kg^{-1}), although increasing the NC response did not significantly alter the inhibitory effect of morphine (1 mg kg^{-1}) ($n = 3$). Yohimbine (0.5 mg kg^{-1}) injected 10 min before morphine did not alter the inhibitory effect of morphine (1 mg kg^{-1}) ($n = 4$). Naloxone (1 mg kg^{-1}) alone had no significant enhancing effect on the NC bronchoconstrictor component evoked by vagal stimulation at the stimulation parameters examined in this experiment.

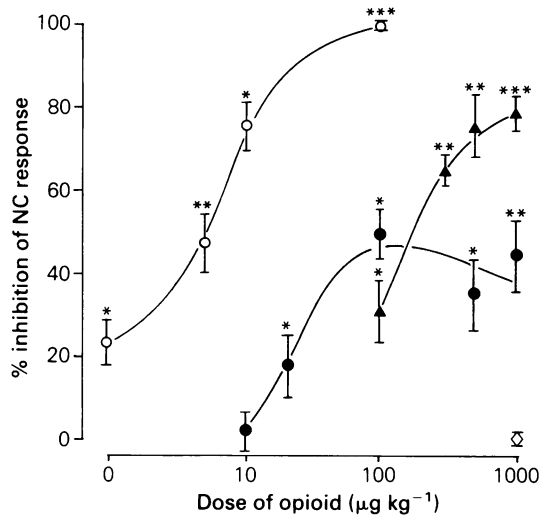

Figure 2 Dose-dependent inhibition of the non-cholinergic (NC) bronchoconstrictor response evoked by vagal stimulation by intravenous DAGOL (○), morphine (▲), DPDPE (●) and U-50,488H (◇). Data are means for 3–6 animals; vertical lines indicate s.e. Significance of inhibition: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Effect of morphine on the responses to acetylcholine and substance P

Exogenous ACh (25 μg kg^{-1}, $n = 4$) increased tracheal pressure to 22.9 ± 2.5 cmH_2O and SP

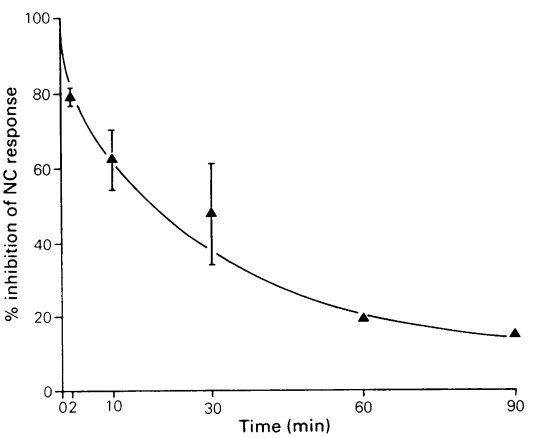

Figure 3 Time course of inhibition of the non-cholinergic (NC) response by morphine (1 mg kg^{-1}). Bilateral vagal stimulation was carried out at 5 different time points (2, 10, 30, 60 and 90 min) after administration of morphine. Data are means and vertical lines indicate s.e. There were 2–5 animals at each time point.

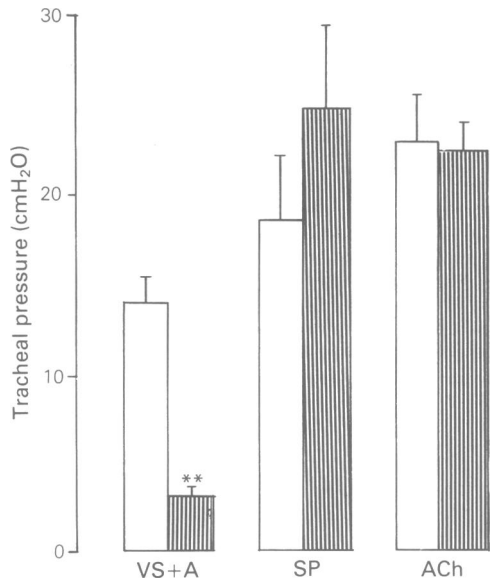

Figure 4 The effect of morphine ($500\,\mu g\,kg^{-1}$) (hatched columns) on bronchoconstriction evoked by vagal stimulation after atropine (VS + A), by exogenous substance P (SP, $25\,\mu g\,kg^{-1}$) and exogenous acetylcholine (ACh, $25\,\mu g\,kg^{-1}$). Open columns represent control responses before morphine. Data are presented as means for 4–5 animals; vertical lines indicate s.e. $^{**}P < 0.01$.

($25\,\mu g\,kg^{-1}$, $n = 4$) to $18.6 \pm 3.5\,cmH_2O$. These responses were not significantly inhibited by morphine ($500\,\mu g\,kg^{-1}$) injected 2 min before ACh or SP (Figure 4).

Effect of selective opioid agonists

Intravenous injection of the μ-selective agonist DAGOL and the δ-selective agonist DPDPE produced an inhibition of the NC bronchoconstrictor response elicited by vagal stimulation with ED_{50} values of $5.4\,\mu g\,kg^{-1}$ and $28\,\mu g\,kg^{-1}$ and maximal inhibitions of 100% and 50%, respectively (Figure 2). However, the κ-selective agonist U-50,488H had no significant inhibitory effect on the NC response.

Effect of opioids on blood pressure

The mean arterial blood pressure (BP) recorded from the left carotid artery before treatment was 47 ± 4 mmHg ($n = 10$). Flushing the artery, to prevent blood clotting in the indwelling cannula, with heparin-saline before experimentation and i.v. saline had no significant or consistent effect on BP. Morphine increased the mean arterial BP at higher doses: at a dose of $1\,mg\,kg^{-1}$ the mean increase was 25 ± 3.3 mmHg ($n = 3$, $P < 0.001$). The increase in BP after morphine injection was transient and, since morphine was injected 2 min before vagal stimulation, BP had returned to control values before the stimulation period. DAGOL increased the mean arterial BP at all concentrations used: at a dose of $100\,\mu g\,kg^{-1}$ the mean increase was 10.4 ± 2.5 mmHg ($n = 3$, $P < 0.05$). The effect of DAGOL on BP was also transient so that the BP had returned to baseline values in the 2 min before stimulation. DPDPE ($1\,mg\,kg^{-1}$) caused a non-significant increase in the mean arterial BP of 4.3 ± 2.4 mmHg ($n = 5$). U-50, 488H ($1\,mg\,kg^{-1}$) decreased the mean blood pressure by 14 ± 2.8 mmHg ($n = 3$, $P < 0.05$).

Discussion

We have demonstrated that morphine inhibits NC bronchoconstriction in the guinea-pig in a dose-dependent manner and that this inhibition can be reversed by the opioid receptor antagonist naloxone. The maximal inhibitory effect with morphine was approximately 80% so that total inhibition was not achieved. The inhibitory effect of morphine was similar in the absence of atropine, indicating that the inhibitory effect may be exerted preferentially on the NC component of bronchoconstriction with some effect on the cholinergic component. These results are consistent with *in vitro* studies in guinea-pig bronchi in which morphine causes a dose-dependent inhibition of the NC contraction induced by electrical field stimulation (Frossard & Barnes, 1987).

NC bronchoconstriction after vagal stimulation may be due to release of neuropeptides such as SP and neurokinins from sensory nerve endings (Lundberg *et al.*, 1983a; Andersson & Grundstrom, 1983). NC bronchoconstriction is inhibited by tachykinin antagonists (Lundberg *et al.*, 1983b) and is not seen in animals pretreated with capsaicin which destroys SP-immunoreactive nerves (Martling *et al.*, 1984). In the present study, NC bronchoconstriction was unaffected by the ganglion blocker hexamethonium, as expected if sensory nerves were involved, and this result is consistent with previous studies (Grundstrom & Andersson, 1985). Morphine had no effect on SP-induced bronchoconstriction, suggesting that its inhibitory effect on NC bronchoconstriction may be due to inhibition of release of SP and other tachykinins from sensory nerve endings. This indicates that opioid receptors may be localized to sensory nerve endings in the airway and is consistent with the demonstration of opioid binding sites on capsaicin-sensitive nerves of the vagus (Nagy *et al.*, 1980). Furthermore, opioids have been found to inhibit SP release from sensory nerves in rat hind paw (Bartho & Szolcsanyi, 1981; Lembeck *et al.*, 1982; Smith & Buchan, 1984;

Lembeck & Donnerer, 1985). Naloxone alone had no significant enhancing effect on NC constriction, indicating that endogenous opioids do not modulate NC bronchoconstriction evoked by the stimulation parameters used in this study. However, in mammalian prevertebral ganglia *in vitro*, the conditioning stimulation given to preganglionic nerves produces a long lasting inhibition of cholinergic transmission which is abolished by naloxone (Konishi *et al.*, 1981). It is, therefore, possible that enkephalin, an endogenous opioid, acts as a transmitter in presynaptic inhibition that controls release of ACh and SP.

It is possible that the effects of morphine might be indirect, perhaps via the release of catecholamines or by interaction with adrenergic nerves. However, these mechanisms are unlikely to operate in our experiments. Thus, after β-adrenoceptor blockade with propranolol, morphine was still able to inhibit NC bronchoconstriction, indicating that the inhibitory effect of morphine was not mediated by stimulation of β-adrenoceptors on airway nerves or on smooth muscle. Morphine caused an increase in blood pressure and is, therefore, unlikely to stimulate catecholamine release via a baroreflex mechanism. The effect of morphine in experimental animals is a lowering of BP which is prevented by bilateral vagotomy (Fennessy & Rattray, 1971). Since α_2-adrenoceptor agonists inhibit NC constriction in the guinea-pig (Grundstrom & Andersson, 1985), it is possible that the inhibitory effect of morphine may be mediated by release of noradrenaline from adrenergic nerves in airways which then acts on α_2-adrenoceptors on sensory nerves. However, the α_2-adrenoceptor antagonist yohimbine did not alter the inhibitory effect of morphine. Therefore, the most likely explanation for our findings is that morphine has a direct effect on opioid receptors on sensory nerve endings in the airway to prevent the release of bronchoconstrictor peptides.

The most potent agonist employed was DAGOL, a μ-selective opioid receptor agonist which exhibits 220 : 1 preference for $\mu : \delta$ receptors (Handa *et al.*, 1981; Pfeiffer *et al.*, 1982; Pasternak, 1986); it inhibited the response by 100%. As was the case with morphine, DAGOL also significantly increased the mean arterial pressure. DPDPE a δ-specific receptor agonist (Mosberg *et al.*, 1983), also inhibited the NC constrictor response but was less potent than DAGOL and gave a maximal inhibition of only 50% even at higher doses. The increase in mean arterial pressure blood pressure produced by DPDPE was not significant. U-50,488H, a κ-selective agonist (Piercey *et al.*, 1982), had no effect on the NC bronchoconstriction evoked by vagal stimulation and there was a significant decrease in the mean arterial blood pressure. Taken together these results suggest that a μ-opioid receptor is involved in the inhibitory response. DPDPE was active to a certain extent although total inhibition could never be attained. This suggests that either a proportion of the NC nerves possess δ-receptors or that DPDPE is acting on μ-receptors to produce the effect. In fact DPDPE has been shown to interact with μ-receptors in the mouse vas deferens (Hirning *et al.*, 1985). Receptors are classically characterized using selective antagonists. However, the only specific antagonist available is ICI 174,864 which is δ-receptor specific but its usefulness is limited by agonist activity (Dray & Nunan, 1984). The κ-agonist, U-50,488H, did not affect the NC response implying that κ-receptors are not involved in the inhibitory effect. More information will be gained with the advent of more specific receptor antagonists.

Axon reflex mechanisms, with release of neuropeptides from sensory nerves in the airway have been implicated in asthma (Barnes, 1986). It is possible that opioids might therefore modulate neurogenic inflammation in the airways and that opioids which are devoid of central effects may have a beneficial effect in asthma. Further studies of this inhibitory opioid receptor-mediated mechanism are therefore warranted.

Supported by grants from Fisons Pharmaceuticals and the Medical Research Council.

References

ANDERSSON, R.G.G. & GRUNDSTROM, N. (1983). The excitatory non-cholinergic, non-adrenergic nervous system of the guinea-pig airways. *Eur. J. Respir. Dis.*, **64**, 141–157.

ATWEH, S.F., MURRIN, L.C. & KUHAR, M.J. (1978). Presynaptic localisation of opiate receptors in the vagal and accessory optic systems: an autoradiographic study. *Neuropharmacol.*, **17**, 65–71.

BARNES, P.J. (1986). Asthma as an axon reflex. *Lancet*, **i**, 242–245.

BARTHO, L. & SZOLCSANYI, J. (1981). Opiate agonists inhibit neurogenic plasma extravasation in the rat. *Eur. J. Pharmacol.*, **73**, 101–104.

BELVISI, M.G., CHUNG, K.F., JACKSON, D.M. & BARNES, P.J. (1987). Opioid control of non-cholinergic bronchoconstriction in guinea-pig *in vivo*. *Br. J. Pharmacol.*, **92**, 595P.

DRAY, A. & NUNAN, L. (1984). Selective δ-opioid receptor antagonism by ICI 174,864 in the central nervous system. *Peptides*, **5**, 1015–1016.

FENNESSY, M.R. & RATTRAY, J.F. (1971). Cardiovascular effects of intravenous morphine in the anaesthetised rat. *Eur. J. Pharmacol.*, **141**, 519–522.

FROSSARD, N. & BARNES, P.J. (1987). μ-Opioid receptors modulate non-cholinergic constrictor nerves in guinea-pig airways. *Eur. J. Pharmacol.*, **141**, 519–522.

GRUNDSTROM, N. & ANDERSSON, R.G.G. (1985). *In vivo* demonstration of α_2-adrenoceptor mediated inhibition of the excitatory non-cholinergic neurotransmission in guinea-pig airways. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 236–240.

HANDA, B.K., LANE, A.C., LORD, J.A.H., MORGAN, B.A., RANCE, M.J. & SMITH, C.F.C. (1981). Analogues of β-LPH 61–64 possessing selective agonist activity at mu opiate receptors. *Eur. J. Pharmacol.*, **70**, 531–540.

HIRNING, L.D., MOSBERG, H.I., HURST, R., HRUBY, V.J., BURKS, T.F. & PORECA, F. (1985). Studies *in vitro* with ICI 174,864, [D-Pen^2, D-Pen^5]-Enkephalin (DPDPE) and [D-Ala^2, NMePhe^4, Gly-ol]-Enkephalin (DAGO). *Neuropeptides*, **5**, 383–386.

JESSELL, T.M. & IVERSEN, L.L. (1977). Opiate inhibit substance P release from trigeminal nucleus. *Nature*, **268**, 549–551.

KONISHI, S., TSUNOO, A. & OTSUKA, M. (1981). Enkephalin as a transmitter for presynaptic inhibition in sympathetic ganglia. *Nature*, **294**, 80–82.

KONZETT, H. & ROSSLER, R. (1940). Versuchsanordnung zu untersuchungen an der bronchialmuskulatur. *Arch. Exp. Path. Pharmak*, **195**, 71–74.

LADURON, P.M. (1984). Axonal transport of opiate receptors in capsaicin-sensitive neurones. *Brain Res.*, **294**, 157–160.

LEMBECK, F., DONNERER, J. & BARTHO, L. (1982). Inhibition of neurogenic vasodilatation and plasma extravasation by substance P antagonists, somatostatin and [D-Met^2, Pro^5] enkephalinamide. *Eur. J. Pharmacol.*, **85**, 171–176.

LEMBECK, F. & DONNERER, J. (1985). Opioid control of the function of primary afferent substance P fibres. *Eur. J. Pharmacol.*, **114**, 241–246.

LUNDBERG, J.M., BRODIN, E. & SARIA, A. (1983a). Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. *Acta Physiol. Scand.*, **119**, 243–252.

LUNDBERG, J.M., SARIA, A., BRODIN, E., ROSELL, S. & FOLKERS, K. (1983b). A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in guinea-pig. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 1120–1124.

MARTLING, C-R., SARIA, A., ANDERSSON, P. & LUNDBERG, J.M. (1984). Capsaicin pretreatment inhibits vagal cholinergic and non-cholinergic control of pulmonary mechanics in the guinea-pig. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **325**, 343–348.

MOSBERG, H.I., HURST, R., HRUBY, V.J., GEE, K., YAMAMURA, H.I., GALLIGAN, J.J. & BURKS, T.F. (1983). Bispenacillamine enkephalins show pronounced delta opioid receptor selectivity. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 5871–5874.

NAGY, J.I., VINCENT, S.R., STAINES, W.M.A., FIBIGER, H.C., REISINE, J.D. & YAMAMURA, H.I. (1980). Neurotoxin action of capsaicin on spinal substance P neurons. *Brain Res.*, **186**, 435–444.

PASTERNAK, G.W. (1986). Multiple mu opiate receptors: biochemical and pharmacological evidence for multiplicity. *Biochem. Pharmacol.*, **35**, 361–364.

PIERCEY, M.F., LAHTI, R.A., SCHROEDER, L.A., EINSPAHR, F.J. & BARSUHN, C. (1982). U-50,488H, a pure kappa receptor agonist with spinal analgesic loci in mouse. *Life Sci.*, **31**, 1197–1200.

PFEIFFER, A., PASI, A., MERHEIN, W. & HERTZ, A. (1982). Opiate binding sites in human brain. *Brain Res.*, **248**, 87–96.

SMITH, T.W. & BUCHAN, P. (1984). Peripheral opioid receptors located on saphenous nerve. *Neuropeptides*, **5**, 217–220.

YOUNG, W.S., WAMSLEY, J.K., ZARBIN, M.A. & KUHAR, M.J. (1980). Opioid receptors undergo axonal flow. *Science*, **210**, 76–78.

(Received February 19, 1988
Revised April 14, 1988
Accepted May 17, 1988)