ELSEVIER

European Journal of Pharmacology 374 (1999) 21–24

ejp

www.elsevier.nl/locate/ejphar

Short communication

Modulation of acetylcholine release from parasympathetic nerves innervating guinea-pig and human trachea by endomorphin-1 and -2

Hema J. Patel [a], Priya Venkatesan [a], James Halfpenny [a], Magdi H. Yacoub [b], Alyson Fox [c], Peter J. Barnes [c], Maria G. Belvisi [d,*]

[a] *Thoracic Medicine, Imperial College School of Medicine at the National Heart and Lung Institute, London SW3 6LY, UK*
[b] *Cardiothoracic Surgery, Imperial College School of Medicine at the National Heart and Lung Institute, London SW3 6LY, UK*
[c] *Novatis Institute for Medical Sciences, London WC1E 6BN, UK*
[d] *Pharmacology Department, Rhône-Poulenc Rorer Research and Development, Rainham Road South, Dagenham, Essex, RM10 7XS, UK*

Received 16 April 1999; accepted 22 April 1999

Abstract

Endomorphin-1 and -2 (μ-opioid receptor agonists) produced a concentration-dependent and naloxone-sensitive inhibition of cholinergic contractile responses in guinea-pig trachea (at 10 μM, 46.1 ± 8.0% and 33.8 ± 8.6%, respectively). Endomorphin-1 and -2 also inhibited electrically-evoked acetylcholine release from cholinergic nerves innervating guinea-pig (at 0.1 μM, 41.8 ± 10.9%; at 1 μM 60.1 ± 6.3%, respectively) and human trachea (at 10 μM, 76.2 ± 18.1%, and 77.7 ± 14.3%, respectively). Naloxone prevented the inhibition by endomorphin-1 and -2 in both guinea-pig and human trachea, suggesting that these peptides can inhibit cholinergic, parasympathetic neurotransmission to the airways via the activation of classical opioid receptors. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Endomorphin-1; Endomorphin-2; Acetylcholine; Airway; Cholinergic neurotransmission; Opioid receptor

1. Introduction

The parasympathetic nerves play a dominant role in the control and regulation of airway smooth muscle tone and mucus secretion in animals and humans (Barnes, 1993). Indirect functional studies suggest that activation of opioid receptors results in inhibition of cholinergic neurotransmission and thus cholinergic contractile responses evoked by electrical field stimulation in human (Belvisi et al., 1992) and guinea-pig (Belvisi et al., 1990) airways. Recently, we confirmed the pre-junctional nature of this response by demonstrating the inhibitory action of [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO) on acetylcholine release from parasympathetic nerves innervating guinea-pig trachea (Patel et al., 1997).

Two endogenous peptides, endomorphin-1 and -2, with high affinity and specificity for the μ-opioid receptor have been isolated from bovine (Zadina et al., 1997) and human (Hackler et al., 1997) brain tissue. A recent study has demonstrated the presence of endomorphin-2 in small diameter primary afferent fibres in rodents and primates (Pierce et al., 1998) and spinally administered endomorphin-1 and -2 inhibit C-fibre evoked neuronal responses (Chapman et al., 1997). However, it is not known whether these peptides modulate peripheral neurotransmission. We have investigated the ability of endomorphin-1 and -2 to modulate cholinergic neurotransmission by measuring cholinergic contractile responses in guinea-pig trachea and by directly measuring electrically-evoked acetylcholine release from parasympathetic nerves innervating guinea-pig and human trachea.

2. Materials and methods

2.1. Preparation of guinea-pig and human trachea

Male Dunkin–Hartley guinea-pigs (full barrier animals; 350–500 g; David Hall, Staffordshire, UK) were cervically dislocated and the trachea removed. Human tracheal smooth muscle was obtained from at least 4 different

* Corresponding author. Tel.: +44-181-919-2075; Fax: +44-181-919-2497; E-mail: maria.belvisi@rp-rorer.co.uk

0014-2999/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(99)00308-8

donors for heart/heart–lung transplantation (patients, aged 21–52 years, three males).

2.2. Measurement of contraction

Transverse segments of epithelium-denuded guinea-pig trachea were suspended between parallel platinum-wire electrodes in 10 ml organ baths containing oxygenated Krebs solution in the presence of indomethacin (10 μM) and isometric contractile responses were measured (Patel et al., 1998). The effect of endomorphin-1 and -2 (0.01–10 μM) on electrically-evoked (40 V, 0.5 ms, 4 Hz for 15 s every 4 min), atropine and tetrodotoxin-sensitive cholinergic contractile responses was investigated. In some experiments, naloxone (10 μM) was added to the tissues for 30 min before, or in some cases, 20 min after incubation (time of maximum effect of endomorphins) with endomorphin-1 or -2 (10 μM each). In a separate series of experiments the effect of endomorphin-1 or -2 (10 μM) was investigated on contractions elicited by the addition of 1 μM acetylcholine, which elicited contractions that were similar in magnitude to those evoked electrically. After three control responses to acetylcholine, endomorphin-1 or -2 (10 μM) was added for 20 min, followed by a final contraction to acetylcholine.

2.3. Measurement of acetylcholine release

Strips of guinea pig or human tracheal smooth muscle were mounted in chambers and superfused with oxygenated Krebs solution in the presence of indomethacin (10 μM). Acetylcholine release was determined by measuring electrically-evoked [^{3}H]overflow (electrical field stimulation parameters: 40 V, 4 Hz, 0.5 ms for 1 min) from epithelium-denuded strips pre-loaded with [^{3}H]choline as described previously (Patel et al., 1995). Electrical field stimulation was applied and 1 ml samples were taken at 1 min intervals for 3 min before, 1 min during, and 3 min

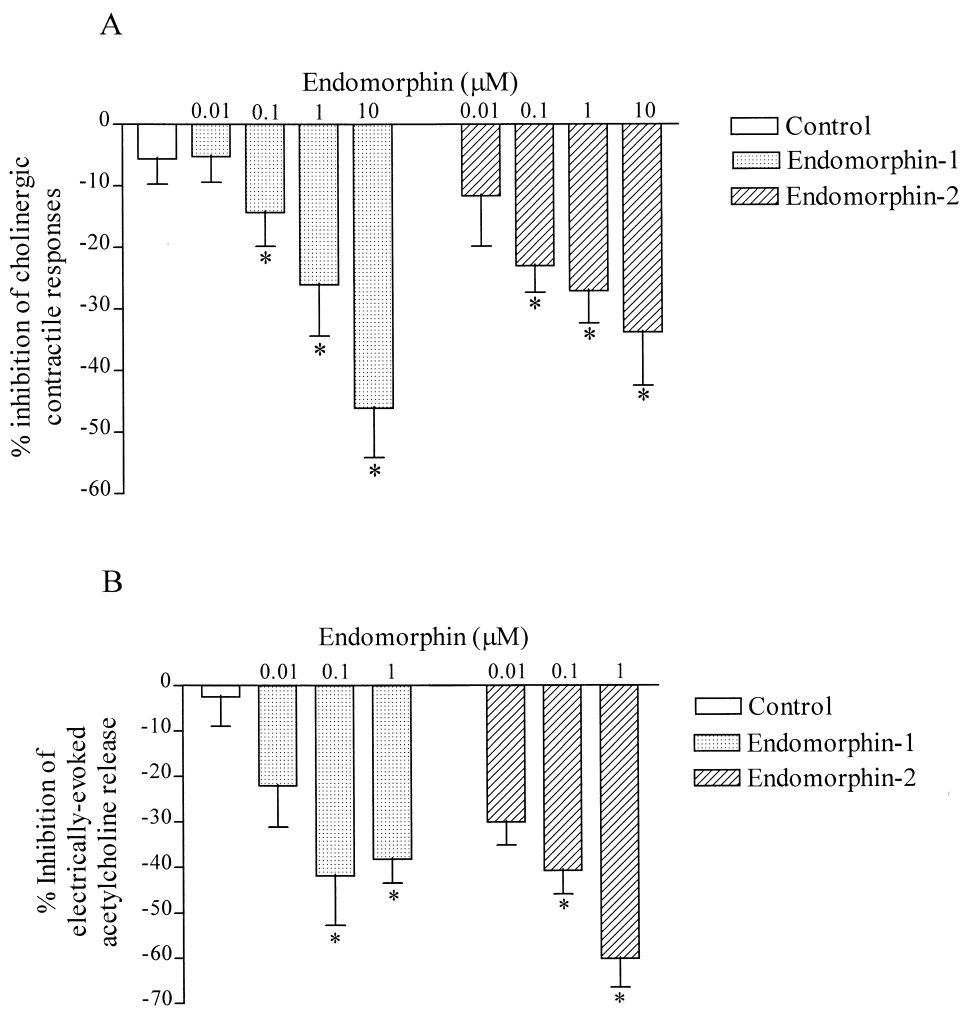

Fig. 1. The effect of endomorphin-1 (0.01–10 μM) and -2 (0.01–10 μM) on electrically-evoked (40 V, 0.5 ms pulse width, 4 Hz for 15 sec every 4 min) cholinergic contractile responses (A) and electrically-evoked (40 V, 0.5 ms pulse width, 4 Hz for 1 min) acetylcholine release (B) from epithelium-denuded guinea-pig trachea. Results are expressed as the mean percentage change from control responses preceding drug administration. Data represent mean ± S.E.M. of at least five independent determinations. * $p < 0.05$; significant inhibition of cholinergic contractile responses or acetylcholine release assessed by Wilcoxon's rank order test for paired data.

after stimulation and 1 ml samples were taken at 5 min intervals outside these times. The tritium release evoked by electrical stimulation under the aforementioned conditions is frequency-dependent and tetrodotoxin-sensitive and, therefore, neuronal in origin (Ward et al., 1993). This has also been demonstrated for guinea-pig trachea under similar conditions (Wessler et al., 1991). Furthermore, the chemical nature of tritium outflow evoked by electrical stimulation of epithelium-denuded tracheal preparations has been shown to be made up of entirely neuronally released [^{3}H]acetylcholine (Wessler et al., 1991, 1995). We therefore assume that [^{3}H]choline overflow represents an accurate index of released [^{3}H]acetylcholine. Endomorphin-1 and -2 (0.01–1 μM) were added to the Krebs solution for 10 min after one control (first) electrical stimulation, followed by a second test electrical stimulation. In some experiments naloxone (10 μM) was added at the beginning of the collection period. The time frame for these experiments was 44 min. In some experiments using human tissue, naloxone (10 μM) was added to the tissues for 30 min after the second (test) stimulation and was followed by a third (second test) stimulation. The time frame for these experiments was 81 min.

2.4. Materials

All drugs were purchased from Sigma (Poole, Dorset, UK) except endomorphin-1 and -2 (purified from bovine brain) which were purchased from Tocris Cookson (Bristol, UK).

2.5. Statistical analysis

In all experiments each tissue acted as its own control. Results obtained before and after drug treatment of predetermined pairs were compared by Wilcoxon's rank order test for paired data. The Kruskal–Wallis non-parametric ANOVA followed by Dunn's test was used for multiple comparisons between tissues.

3. Results

Both endomorphin-1 and -2 (0.01–10 μM) inhibited cholinergic contractile responses in guinea-pig trachea in a concentration-dependent manner (Fig. 1A) while having no effect on contractions evoked by exogenous acetylcholine (data not shown). The inhibitory action of endomorphin-1 (10 μM) and -2 (10 μM) on cholinergic contractile responses was significantly reversed by the addition of naloxone (10 μM; 68.5 ± 6.1%, $n = 5$, $p < 0.05$ and 86.2.3 ± 6.0% reversal, $n = 5$, $p < 0.05$, respectively) and was abolished by pre-treatment of the tissues with naloxone (10 μM; 0.6 ± 2.2% inhibition, $n = 5$ and 4.1 ± 4.4% inhibition, $n = 5$, respectively), under conditions where naloxone (10 μM) alone had no effect (4.8 ± 2.8% inhibition, $n = 3$). Endomorphin-1 inhibited electrically-evoked acetylcholine release from guinea-pig trachea in a concentration-dependent manner as did endomorphin-2 (Fig. 1B). Naloxone (10 μM) alone had no effect (5.17 ± 10.3% inhibition, $n = 8$, NS). However, pre-treatment of tissues with naloxone (10 μM for 20 min) abolished the inhibitory effect of both endomorphin-1 (at 1 μM, 10.7 ± 32.4% inhibition, $n = 7$, NS) and -2 (at 1 μM, 12.08 ± 7.48% inhibition, $n = 8$, NS). Furthermore, endomorphin-1 and -2 inhibited acetylcholine release from cholinergic nerves innervating human trachea (at 1 μM, 64.7 ± 11.7%, $n = 4$, $p < 0.05$ and 64.1 ± 15% inhibition, $n = 3$, $p < 0.05$, respectively; at 10 μM, 76.2 ± 18.1%, $n = 3$, $p < 0.05$ and 77.7 ± 14.3% inhibition, $n = 3$, $p < 0.05$). As in guinea-pig tissue, naloxone (10 μM) alone had no effect (5.8 ± 9.0% inhibition, $n = 3$). However, the inhibitory action of endomorphin- 1 and -2 (both at 10 μM) was reversed by naloxone (109.7 ± 12.8%, reversal, $n = 3$, $p < 0.05$ and 109.3 ± 24 7% reversal, $n = 3$, $p < 0.05$, respectively; see Fig. 2A and B for representative profiles).

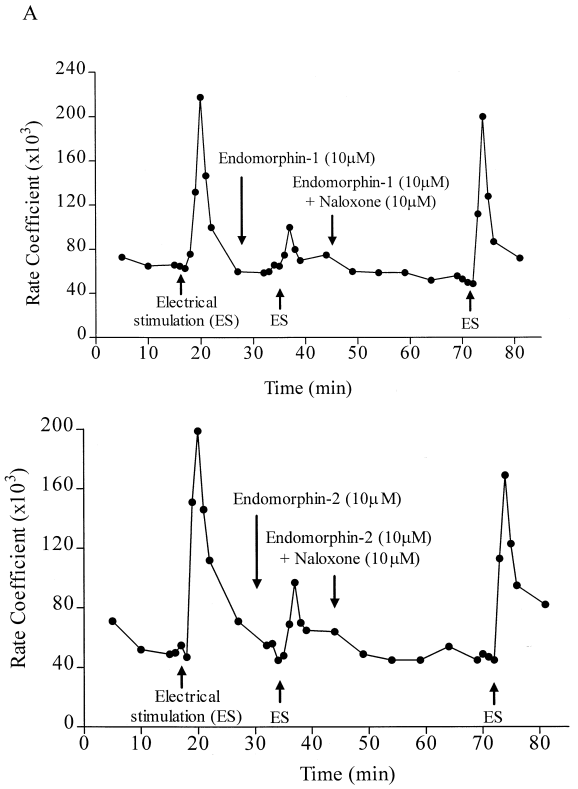

Fig. 2. Profile of the effect of naloxone (10 μM) on the inhibitory action of (A) endomorphin-1 (10 μM) and (B) -2 (10 μM) on electrically-evoked (40 V, 0.5 ms pulse width, 4 Hz for 1 min) acetylcholine release from a single, epithelium-denuded human tracheal strip. Results are expressed as the rate coefficient ($\times 10^3$), which is a measure of the fractional [^{3}H] release plotted against time (min).

4. Discussion

These results provide persuasive evidence for the first time that endomorphin-1 and -2 modulate cholinergic neu-

rotransmission in a peripheral tissue. Endomorphin-1 and -2 suppressed electrically-evoked cholinergic contractile responses without affecting tension elicited by exogenous acetylcholine in guinea-pig trachea and they inhibited electrically evoked acetylcholine release from parasympathetic nerve terminals innervating human and guinea-pig trachea. Naloxone abolished the inhibitory action of endomorphin-1 and -2 on both contractile responses and acetylcholine release in guinea-pig airways and on acetylcholine release in human airways. The level of inhibition of acetylcholine release observed at 1 μM for both endomorphin-1 and -2 in guinea-pig trachea is not as great as that seen with the M2-muscarinic receptor agonist, oxotremorine M (at 1 μM ~ 77% inhibition, Patel et al., 1995). However, they are similar to that seen with DAMGO at 1 μM (~ 36% inhibition, Patel et al., 1997). In human trachea, oxotremorine M (1 μM) inhibits acetylcholine release by approximately 73% (Patel et al., 1995), which is similar to the effects of endomorphins described in the present study. It appears then, that as well as there being an autoregulatory feed-back mechanism to control the release of acetylcholine, endogenous endomorphins, if they are present in the airways, may also limit acetylcholine release and hence vagally-mediated bronchoconstriction in human trachea. The naloxone-sensitive nature of the response elicited by the endomorphins is in agreement with other studies demonstrating significant naloxone-sensitive vasodilator activity in the systemic vascular bed of the rabbit (Champion et al., 1997a) and in the hindquarters vascular bed of the rat (Champion et al., 1997b). In addition, the inhibitory effects of spinal endomorphin-1 and -2 on evoked dorsal horn neuronal responses in the rat were partially reversed by naloxone (Chapman et al., 1997).

In conclusion, this data suggests that the μ-opioid receptor ligands endomorphin-1 and -2 inhibit cholinergic neurotransmission pre-junctionally in guinea-pig and human trachea through an interaction with 'classical' (naloxone-sensitive) receptors. Theoretically, peripherally acting μ-opioid receptor agonists, devoid of central actions, might have therapeutic potential in the treatment of cholinergic reflex bronchoconstriction and mucus secretion.

Acknowledgements

Hema Patel was supported by the Wellcome Trust and Priya Venkatesan by a ROPA award from the Medical Research Council.

References

Barnes, P.J., 1993. Muscarinic receptor subtypes in airways. Life Sci. 52, 521–527.

Belvisi, M.G., Stretton, C.D., Barnes, P.J., 1990. Modulation of cholinergic neurotransmission in guinea-pig airways by opioids. Br. J. Pharmacol. 100, 131–137.

Belvisi, M.G., Stretton, C.D., Verleden, G.M., Ledingham, S.J.L., Yacoub, M.H., Barnes, P.J., 1992. Inhibition of cholinergic neurotransmission in human airways by opioids. J. Appl. Physiol. 72, 1096–1100.

Champion, H.C., Zadina, J.E., Kastin, A.J., Hackler, L., Ge, L.-J., Kadowitz, P.J., 1997a. The endogenous mu-opioid receptor agonists endomorphins 1 and 2 have novel hypotensive activity in the rabbit. Biochem. Biophys. Res. Comm. 235, 567–570.

Champion, H.C., Zadina, J.E., Kastin, A.J., Kadowitz, P.J., 1997b. The endogenous μ-opioid agonists, endomorphin-1 and -2, have vasodilator activity in the hindquarters vascular bed of the rat. Life Sci. 61, 409–415.

Chapman, V., Diaz, A., Dickenson, A.H., 1997. Distinct inhibitory effects of spinal endomorphin-1 and endomorphin-2 on evoked dorsal horn neuronal responses in the rat. Br. J. Pharmacol. 122, 1537–1539.

Hackler, L., Zadina, J.E., Ge, L.J., Kastin, A.J., 1997. Isolation of relatively large amounts of endomorphin-1 and endomorphin-2 from human brain cortex. Peptides 18, 1635–1639.

Patel, H.J., Barnes, P.J., Takahashi, T., Tadjkarimi, S., Yacoub, M.H., Belvisi, M.G., 1995. Evidence for prejunctional muscarinic autoreceptors in human and guinea-pig trachea. Am. J. Respir. Crit. Care Med. 152, 872–878.

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.

Patel, H.J., Giembycz, M.A., Keeling, J.E., Barnes, P.J., Belvisi, M.G., 1998. Inhibition of cholinergic neurotransmission in guinea-pig trachea by NS1619, a putative activator of large-conductance, calcium-activated potassium channels. J. Pharmacol. Exp. Ther. 286, 952–958.

Pierce, T.L., Grahek, M.D., Wessendorf, M.W., 1998. Immunoreactivity for endomorphin-2 occurs in primary afferents in rats and monkey. Neuroreport 16, 385–389.

Ward, J.K., Belvisi, M.G., Fox, A.J., Miura, M., Tadjkarimi, S., Yacoub, M.H., Barnes, P.J., 1993. Modulation of cholinergic neural bronchoconstriction by endogenous nitric oxide and vasoactive intestinal peptide in human airways in vitro. J. Clin. Invest. 92, 736–742.

Wessler, I., Klein, A., Pohan, D., Maclagan, J., Racké, K., 1991. Release of [^{3}H]acetylcholine from the isolated rat or guinea-pig trachea evoked by preganglionic nerve stimulation; a comparison with transmural stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 404–411.

Wessler, I., Bender, H., Härle, P., Höhle, K., Kirdorf, G., Klapproth, H., Reinheimer, T., Rícný, J., Schnieppi-Mendelssohn, K.E., Racké, K., 1995. Release of [^{3}H]acetylcholine in human isolated bronchi: effect of indomethacin on muscarinic autoinhibition. Am. J. Respir. Crit. Care Med. 151, 1040–1046.

Zadina, J.E., Hackler, L., Ge, L.J., Kastin, A.J., 1997. A potent and selective endogenous agonist for the μ-opiate receptor. Nature 386, 499–502.