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SPECIAL REPORT

Naloxone blocks endomorphin-1 but not endomorphin-2 induced inhibition of tachykinergic contractions of guinea-pig isolated bronchus

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The recently identified endogenous agonists on the μ-opioid-receptor (μOR), endomorphin-1 (EM-1) and endomorphin-2 (EM-2), induce a concentration dependent inhibition of electrical field stimulation (EFS)-induced tachykinin-mediated contractions of the guinea-pig bronchus (ED_{50s} <10 nM for both compounds). Surprisingly, only endomorphin-1 effects could be blocked by naloxone (10 μM), whereas endomorphin-2 effects were not affected by specific antagonists for the μ-, κ-, and δ-opioid-receptor.

Keywords: Endomorphin; opioid receptors; sensory nerves; tachykinins; guinea-pig

Abbreviations: DAMGO, [D-Ala2, N-Me-Phe4, Gly-ol5]-enkephalin; EFS, electrical field stimulation; EM, endomorphin; NK, neurokinin; NKA, neurokinin A; OR, opioid receptor; ORL, opioid receptor like; crc, concentration response curve

Introduction Endomorphin-1 (EM-1) and -2 (EM-2) are two recently discovered neuropeptides with potent and selective activity at the μ-opioid-receptor (μ-OR; Zadina *et al*., 1997). Selective endogenous ligands for the δ-OR (enkephalins), the κ-OR (dynorphin) and the opioid-receptor like 1 (ORL1; nociceptin/orphanin FQ) have been identified previously (for review see Dhawan *et al*., 1996). However none of the previously known endogenous opioids displayed selectivity for the μ-OR. The amino acid sequence of the novel opioid peptides (endomorphin-1: Tyr-Pro-Trp-Phe, endomorphin-2: Tyr-Pro-Phe-Phe) differs structurally from the N-terminal sequence of classical opioids (Tyr-Gly-Gly-Phe-X-) and from the recently discovered opioid nociceptin/orphanin FQ (Phe-Gly-Gly-Phe-X-; Meunier *et al*., 1995, Reinscheid *et al*., 1995). The presence of endomorphins as endogenous peptides has been demonstrated in the central nervous system of the rat by HPLC-profiles (Zadina *et al*., 1997) and recently also for the spinal cord and the peripheral nervous system by immunohistochemistry (Martin-Schild *et al*., 1997).

In the airways, opioids have been shown to modulate release of mediators from parasympathetic and from sensory nerve fibres by a naloxone sensitive mechanism (Belvisi *et al*., 1990; Bartho *et al*, 1987). In addition, it has been shown that the ORL1-ligand nociceptin has potent, naloxone-insensitive inhibitory effects on mediator release in the airways (Patel *et al*., 1997; Fischer *et al*., 1998). In this study, we have determined the effects of EM-1 and EM-2 on electrical field stimulation (EFS)-induced tachykinergic contractions of guinea-pig isolated bronchi and role of the μ-OR.

Methods The methods used for functional and pharmacological studies have been described in detail previously (Ellis & Undem, 1994). In brief, left or right main stem bronchi of male Hartley guinea-pigs were isolated, placed in organ chambers in modified Krebs' buffer containing atropine (1 μM), propanolol (1 μM) and indomethacin (3 μM) and attached to force transducers. The tissues were stretched to 1 g of resting tension. A Grass (Quincy, MA, U.S.A.) model 7 polygraph was used to record isometric tension. The tissues were stimulated in an electrical field current using a Grass S48 stimulator and a StimuSplitter (Med Lab Instruments, Fort Collins, CO, U.S.A.). Stimulation parameters were 20 V, 1 msec and 5 Hz for 15 s. This resulted in a current of about 300 mA. This technique results in reproducible contractions, that are blocked by either tetrodotoxin, or a combination of NK-1 (CP 9994, 1 μM) and NK-2 (SR 10453, 1 μM) receptor antagonists (Ellis & Undem, 1994). Thus they are termed neurally evoked tachykinin-mediated contractions. This was repeated at 20 min intervals. In untreated tissues there was no difference in the magnitude of five consecutive responses. Control responses were defined as the average of the contractions to the first two stimulations. Increasing concentrations of EM-1 or EM-2 (PolyPeptide Laboratories, Wolfenbüttel, Germany), (or vehicle) were applied to the organ bath 15 min prior to the stimulation and a concentration response curve (c.r.c.) was calculated for each of the peptides. The inhibitory effect of the endomorphins was expressed as a percentage of the control response. In separate studies various opioid receptor antagonists were added to the tissue bath 30 min prior to the first control response; i.e. over 60 min prior to applying the endomorphin. In a final set of studies, the effect of EM-1 and EM-2 on smooth muscle contraction induced by capsaicin were obtained in guinea-pig isolated bronchi, in the presence or absence of the endomorphin. There was no difference in the response of the right vs the left bronchus to capsaicin. In each experiment, one bronchus was treated with the endomorphin, and the contralateral served as control. For the NKA study, three contiguous segments of the distal trachea were used with one segment serving as control and the other two receiving either EM-1 or EM-2 for 30 min prior to the addition of NKA.

The maximum contraction was obtained by addition of barium chloride (30 mM) to the tissue bath at the end of each

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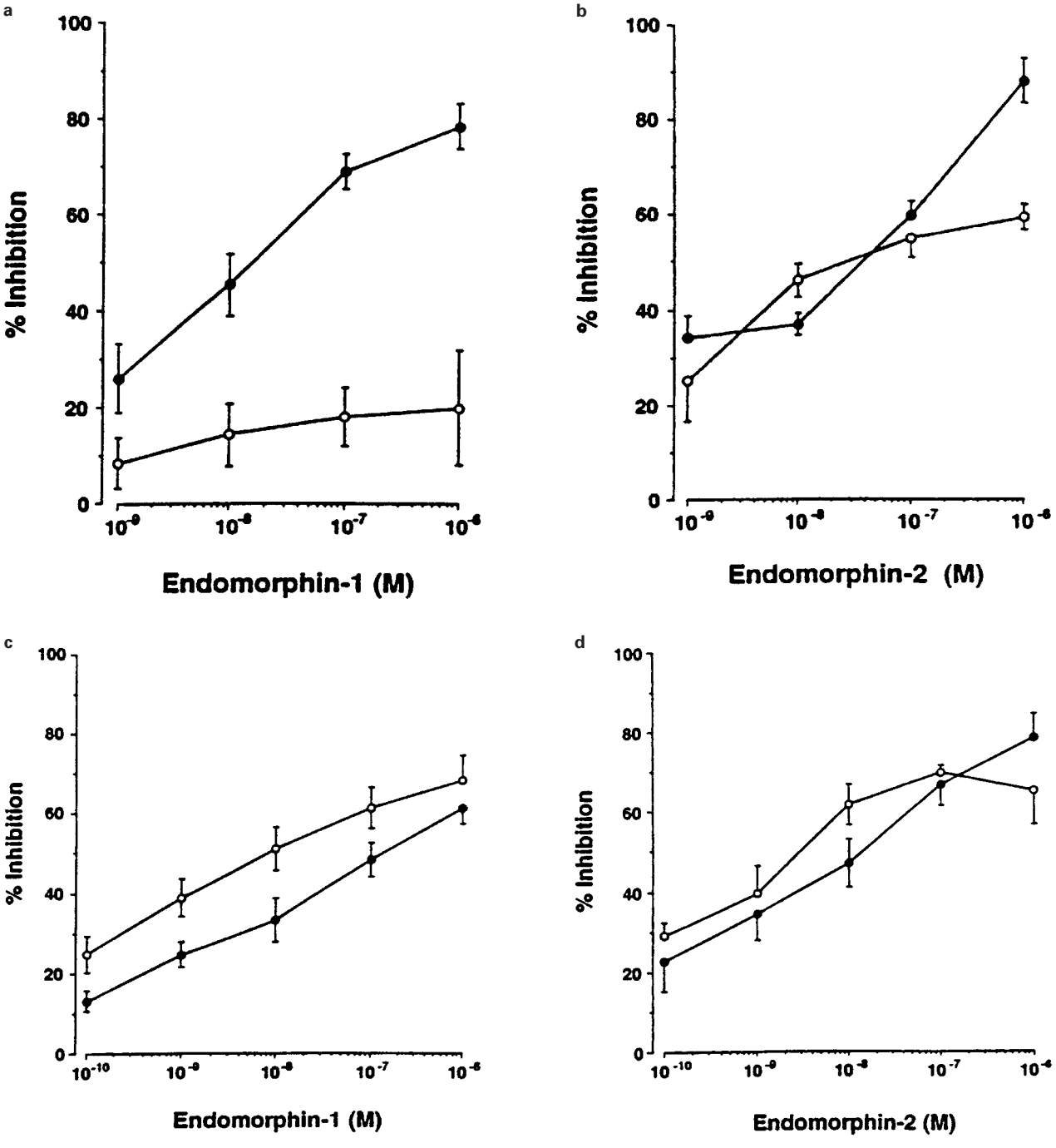
experiment. The data of mean responses between two treatments were analysed using Student's *t*-test for paired comparisons. In the antagonist studies, the data of multiple mean values were first compared by an ANOVA calculation. None of the antagonists had a significant effect on the amount of contraction caused by barium chloride.

Results

Both EM-1 and EM-2 were potent inhibitors of the EFS-induced contractions of the guinea-pig bronchus. EM-1 and EM-2 at 1 μM (largest concentration studied) produced a significant inhibition ($P<0.05$) of EFS-induced tachykinergic contractions by $62\pm4.8\%$ ($n=11$) and $76.8=4.9\%$ ($n=10$), respectively. Taking these values as the maximum response in each experiment, the respective $-\log$ M EC_{50} for EM-1 and EM-2 averaged 8.4 ± 0.3 and 8.6 ± 0.3. The inhibitory effect of EM-1 was blocked by naloxone (10 μM; Figure 1a). Naloxone, by contrast, did not block the inhibitory effect of EM-2 (Figure 1b). The δ-OR antagonist naltrindole and the κ-OR antagonist nor-binaltorphimine (both at 10^{-5} M, Figure 1c and d) had no effect on the response to either EM-1 or EM-2.

Contractions of the guinea-pig isolated bronchus evoked by capsaicin (Figure 1e and f) were not affected by EM1 or EM2 (1 μM). Similarly, contractions of isolated tracheal strips by NKA were not affected by either endomorphin (Figure 1g).

Discussion As shown in several earlier studies, EFS-induced contractions of the guinea-pig bronchus in the presence of atropine and propranolol can be blocked by NK2-receptor specific antagonists and are therefore considered to be



tachykinergic (Ellis & Undem, 1994). EM-1 and EM-2 efficiently inhibit this EFS-induced tachykinergic contraction of the isolated guinea-pig bronchus with potencies similar to that of nociceptin (Fischer *et al*., 1998). As compared to the synthetic μ-agonist DAMGO (Belvisi *et al*., 1990) the endogenous agonists EM-1 and EM-2 appear to be at least ten times more potent.

The effects of EM-1 and EM-2 are most likely mediated by an inhibition of tachykinin release (prejunctional effect), since the contractile response to both exogenously applied NKA was unaffected. Also supporting a prejunctional mechanism of action is the observation that the endomorphins did not inhibit capsaicin-induced contractions. Capsaicin is similar to EFS in this design in that the contractions can be blocked by NK-1 and NK-2 receptor antagonists (Ellis & Undem, 1994). Capsaicin differs from EFS, however, in that it is insensitive to tetrodotoxin (Canning & Undem, 1994). Thus, as we have previously found with nociceptin and other neuromodulators (Fischer *et al*., 1998; Undem *et al*., 1994), the effect of endomorphins appears to be selective for action potential-driven tachykinin release in this tissue.

As predicted from the discovery of EM-1 as endogenous agonist of the μ-OR (Zadina *et al*., 1997), its inhibitory effect on the EFS-induced tachykinergic contractions to EFS was blocked by naloxone, whereas antagonists of other opioid receptors, were ineffective. Surprisingly, however, the inhibitory effects of EM-2, which has also been designated as agonist on the μ-OR, were not blocked by naloxone or any other OR-antagonist tested. In previous pharmacological *in-vivo* studies on endomorphin effects, the effects of both EM-1 and EM2 could be blocked by naloxone in the rabbit and in the rat (Champion *et al*. 1997; Stone *et al*. 1997). However, differential effects between EM-1 and EM-2 have also been noted in dorsal horn neuronal responses in the rat (Chapman *et al*. 1997).

In conclusion, EM-1 and EM-2 are potent inhibitors of electrical field stimulation-induced tachykinergic contractions of guinea-pig isolated bronchi. The mechanism appears to be

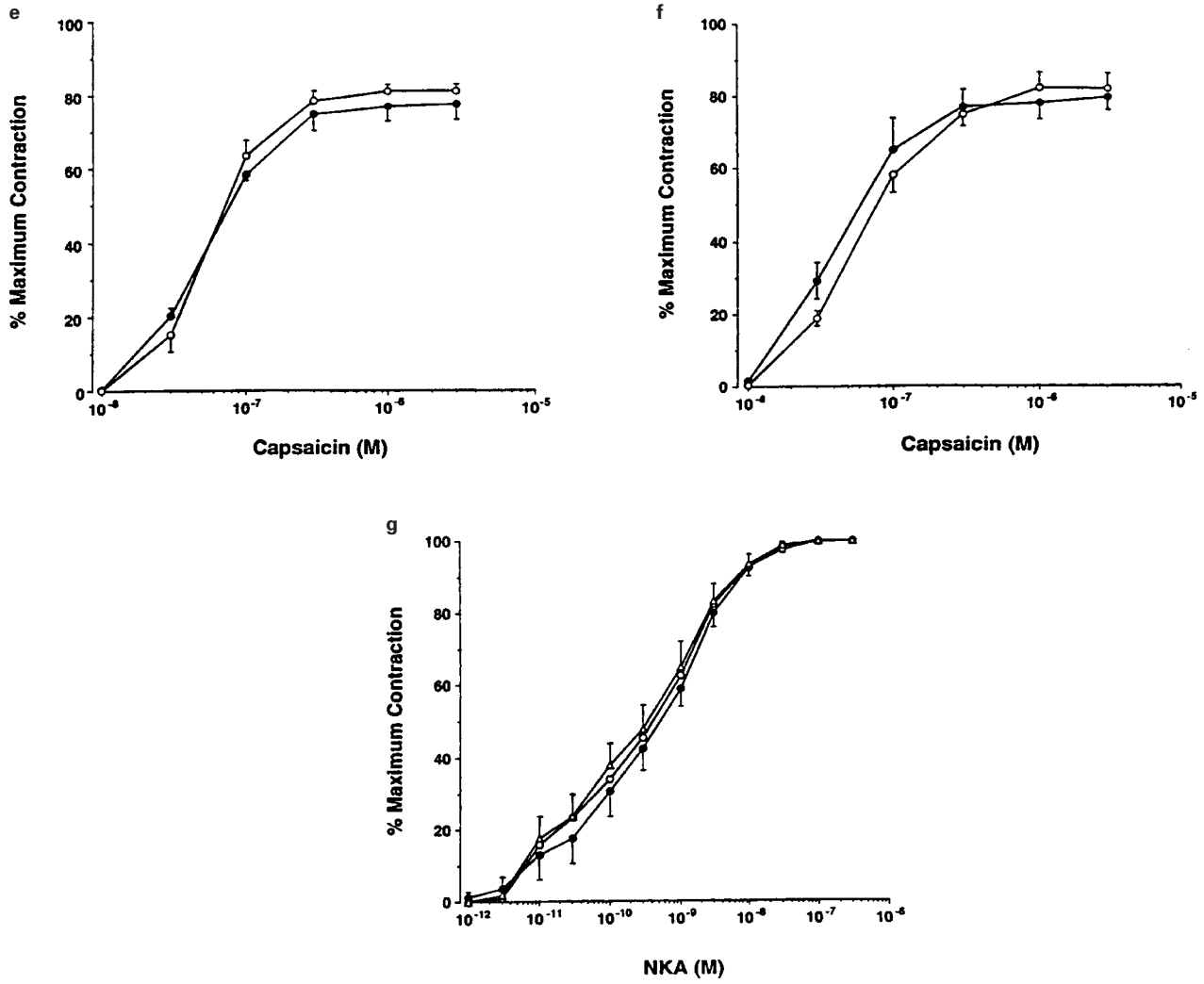


Figure 1 (a,b) CRC of EM-1 (a) and EM-2 (b) induced inhibition of EFS-induced tachykinergic constractions of guiniea-pig bronchus in the absence or presence of naloxone (10^{-5} M). (c,d) CRC of EM-1 (c) and EM-2 (d) induced inhibition of EFS-induced tachykinergic contaction of guinea-pig bronchus in the absence or presence of naltrindole, norbinaltorphimine (c) and naloxone (10^{-5} M) (d). The differences between absence or presence of combined antagonists are not statistically significant. (e,f) Capsaicin induced bronchoconstriction in the absence or presence of EM-1 (e) or EM-2 (f). (g) Contractions of guinea-pig bronchus induced by exogenously administered NKA in the absence or presence of EM-1 or EM-2. Data are given as a percentage of the control response in a–d and of maximal contractions in e–g. They are expressed as means±s.e.mean.

prejunctional in nature. The μ-OR receptors are involved in the response to EM-1. The effect of EM-2, however, may be due to receptors other than, or in addition to, the classical opioid receptors.

References

BARTHO, L., AMANN, R., SARIA, A., SZOLCSANYI, J. & LEMBECK, F. (1987). Peripheral effects of opioid drugs on capsaicin-sensitive neurones of the guinea-pig bronchus and rabbit ear. *Naunyn Schmiedebergs Arch. Pharmacol.,* **336,** 316 – 320.

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.

CANNING, B.J. UNDEM, BJ. (1994). Evidence that antidromically stimulated vagal afferents activate inhibitory neurones innervating guinea-pig trachealis. *J. Physiol.,* **480,** 613 – 625.

CHAMPION, H.C., ZADINA, J.E., KASTIN, A.J., HACKLER L., GE, L.J. & KADOWITZ, P.J. (1997). The endogenous mu-opioid receptor agonists endomorphins 1 and 2 have novel hypotensive activity in the rabbit. *Biochem. Biophys. Res. Comm.,* **235,** 567 – 570.

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.

DHAWAN, B.N., CESSELIN, F., RAGHUBIR, R.,REISINE, T., BRADLEY, P.B., PORTOGHESE, P.S. & HAMON, M. (1996). International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol Rev.,* **48,** 567 – 592.

ELLIS, J.L. & UNDEM, B.J. (1994). Inhibition by capsazepine of resiniferatoxin- and capsaicin-induced contractions of guinea-pig trachea. *J. Pharmacol. Exp. Ther.,* **268,** 85 – 89.

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

MARTIN-SCHILD, S., ZADINA, J.E., GERALL, A.A., VIGH, S. & KASTIN A.J. (1997). Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. *Peptides,* **18,** 1641 – 1649.

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, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor like ORL_1 receptor. *Nature,* **377,** 532 – 535.

PATEL, P.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.

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.

STONE, L.S., FAIRBANKS, C.A., LAUGHLIN, T.M. NGUYEN, H.O., BUSHY, T.M., WESSENDORF, M.W. & WILCOX, G.L. (1997). Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2. *NeuroReport,* **8,** 3131 – 3135.

UNDEM, B.J. MEEKER, S.N., CHEN, J. (1994). Inhibition of neurally mediated nonadrenergic, noncholinergic contractions of guinea pig bronchus by isozyme-selective phosphodiesterase inhibitors. *J. Pharmacol. Exp. Ther.,* **271,** 811 – 817.

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.

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