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SPECIAL REPORT

Naloxone-insensitive inhibition of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by the novel opioid, nociceptin

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The novel peptide, nociceptin and the μ-opioid agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) produced a concentration-dependent inhibition of electrical field stimulation (EFS)-evoked release of acetylcholine (ACh) from cholinergic nerves innervating guinea-pig trachea. The non-selective opioid receptor antagonist, naloxone, did not antagonize the inhibitory action of nociceptin under conditions where the inhibition of ACh release evoked by DAMGO was completely reversed. It is suggested that DAMGO and nociceptin can inhibit cholinergic, parasympathetic neurotransmission to the airways via the activation of classical (naloxone-sensitive) and novel (naloxone-insensitive) opioid receptors, respectively.

Keywords: Cholinergic neurotransmission; opioids; nociceptin; airways; parasympathetic nerves

Introduction Nociceptin (Meunier *et al*., 1995) or orphanin FQ (Reinscheid *et al*., 1995) is a newly discovered heptadecapeptide which has some homology to the dynorphin family of peptides but lacks the N-terminal tyrosine residue essential for activity at the μ, δ and κ opioid receptors. Nociceptin is thought to be the endogenous ligand for the orphan or opioid receptor like-1 (ORL_1) receptor which has high homology at the amino acid level to that of opioid receptors (Bunzow *et al*., 1994) and, like them, is negatively coupled to adenylyl cyclase (Mollereau *et al*., 1994) via a pertussis toxin-sensitive G-protein (Connor *et al*., 1996). However, despite these similarities, ORL_1 has a pharmacology different to classical opioid receptors. Behavioural studies have demonstrated that nociceptin has central hyperalgesic properties since it increases perception to pain *in vivo* (Reinscheid *et al*., 1995; Meunier *et al*., 1995), but like opioids, it is antinociceptive at the spinal level *in vivo* (Stanfa *et al*., 1996) and has an inhibitory action on peripheral sensory neurotransmission *in vitro* (Giuliani & Maggi, 1996).

Indirect functional studies suggest that activation of μ-opioid receptors inhibits cholinergic neurotransmission in guinea-pig (Belvisi *et al*., 1990) and human airways (Belvisi *et al*., 1992) via a pre-junctional mechanism. We have investigated whether nociceptin also has the ability to modulate cholinergic neurotransmission by measuring directly electrical field stimulation (EFS)-induced ACh release from parasympathetic nerves innervating guinea-pig trachea, and have compared it with the μ-opioid agonist, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO).

Methods Male Dunkin-Hartley guinea-pigs (300–500 g) were killed by cervical dislocation and the trachea was removed. Strips of tracheal smooth muscle were mounted in chambers and super perfused with oxygenated Krebs solution in the presence of indomethacin (10 μM). ACh release was determined by measuring ^{3}H-overflow evoked by electrical field stimulation (EFS: 40 V, 4 Hz, 0.5 ms for 1 min) from epithelium-denuded tracheal strips pre-loaded with [^{3}H]-choline as described previously (Patel *et al*., 1995). EFS was applied and 1 ml samples were taken at 1 min intervals for 3 min before, 1 min during, and 3 min after stimulation and 5 min intervals outside these times. The μ-opioid agonist, DAMGO (0.1 μM–10 μM) or nociceptin (0.1 μM–3 μM) were added to the Krebs solution after one control EFS for 10 min, followed by a second EFS. In some cases this was followed by the addition of naloxone (10 μM) for 30 min followed by a third EFS.

All drugs were obtained from the Sigma Chemical Company (Poole, Dorset) except nociceptin which was purchased from Tocris Cookson (Bristol, U.K.).

Results Nociceptin (0.1–3.0 μM) inhibited EFS-evoked ACh release in a concentration-dependent manner by $22.8 \pm 8.8\%$ ($n=8$), $37.0 \pm 5.7\%$ ($n=8$) and $50.5 \pm 10.8\%$ ($n=6$) at 0.1, 1 and 3 μM, respectively (Figure 1a). DAMGO similarly inhibited cholinergic neurotransmission at 1 and 10 μM by $35.9 \pm 4.1\%$ ($n=14$) and $42.3 \pm 6.7\%$ ($n=6$), respectively (Figure 1a). The classical non-selective opioid receptor antagonist, naloxone (10 μM), failed to antagonise the inhibitory action of nociceptin (1 μM), under conditions where the inhibition of EFS-induced ACh release evoked by DAMGO (1 μM) was reversed by $93.9 \pm 30.4\%$ ($n=5$; see Figure 1b and c for representative profiles).

Discussion These results demonstrate for the first time that nociceptin modulates cholinergic neurotransmission in a peripheral tissue. Nociceptin, like the μ-opioid agonist, DAMGO, inhibited ACh release from parasympathetic nerve terminals in guinea-pig trachea. However, the finding that naloxone antagonized the inhibitory action of DAMGO but failed to affect the inhibitory action of nociceptin under identical experimental conditions suggests that nociceptin lacks activity at the μ, δ and κ opioid receptors. This conclusion is in agreement with other studies where nociceptin has been shown to be insensitive to naloxone (Giuliani & Maggi, 1996). The abundance of ORL_1 mRNA in human, mouse (Mollereau *et al*., 1994) and rat brain (Bunzow *et al*., 1994), as well as in peripheral organs such as intestine, vas deferens and spleen suggests that nociceptin may play an important neuromodulatory role in these tissues. However, whilst it is tempting to speculate that nociceptin is acting on ORL_1 receptors, confirmation of this possibility will have to await the discovery of selective antagonists.

In conclusion, this study suggests that μ-opioids and nociceptin inhibit cholinergic neurotransmission in guinea-pig trachea prejunctionally through an interaction with classical (naloxone-sensitive) and novel (naloxone-insensitive, possibly

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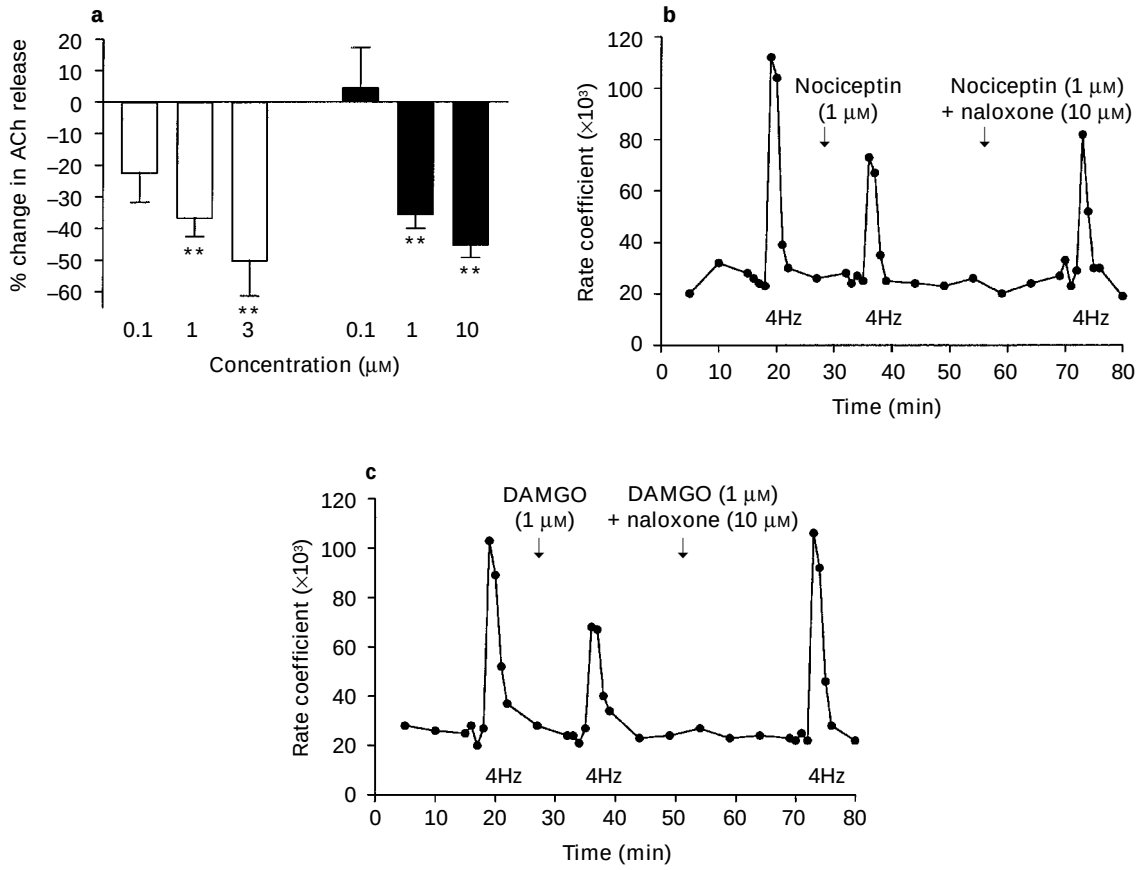

Figure 1 (a) The effect of nociceptin (open columns) (0.1 μM – 3 μM) and DAMGO (solid columns) (0.1 μM – 10 μM) on ACh release from epithelium-denuded guinea-pig tracheal strips evoked by EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min). Results are expressed as the mean percentage change of the response after drug administration compared to the first control stimulation. Data represent mean ± s.e.mean of six to eight independent determinations. $^{**}P<0.01$; significant inhibition of ACh release assessed by Student's paired t test. (b and c) Profile of EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-evoked ACh release from a single guinea-pig tracheal strip showing the effect of nociceptin (1 μM) and DAMGO (1 μM), respectively, before and after the addition of naloxone (10 μM). Results are expressed as a rate coefficient ($\times 10^3$) which is a measure of the fractional ^{3}H-release plotted against time (min).

ORL_1) receptors, respectively. Theoretically, nociceptin might have therapeutic potential in the treatment of cholinergic reflex bronchoconstriction and mucus secretion if it lacks the central effects ascribed to classical opioids.

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