British Journal of Pharmacology (1996) 118, 1567-1569

SPECIAL REPORT

Inhibition of tachykinin release from peripheral endings of sensory nerves by nociceptin, a novel opioid peptide

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The novel heptadecapeptide opioid, nociceptin, produced a concentration-dependent (EC_{50} 28 nM) suppression of the inotropic response of the guinea-pig isolated renal pelvis to electrical stimulation, a response mediated by release of tachykinins from sensory nerves. Nociceptin did not affect the response to neurokinin A, indicating a prejunctional site of action on tachykininergic nerves. The effect of nociceptin was unchanged in the presence of the μ, δ and κ opioid receptor antagonists, naloxone, naltrindole and nor-binaltorphimine.

Keywords: Nociceptin; opioid peptides; sensory neurones; tachykinins; prejunctional action

Introduction Recently, two groups (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995) reported the isolation of a novel heptadecapeptide which shows an opioid-like NH_2 terminal tetrapeptide sequence: the novel peptide, termed nociceptin (Meunier *et al.*, 1995) or orphanin FQ (Reinscheid *et al.*, 1995) binds with nanomolar affinity to the opioid-like G-protein-coupled orphan receptor ORL_1 (Mollereau *et al.*, 1994; Wang *et al.*, 1994). Nociceptin is devoid of opioid-like analgesic activity while being endowed with central hyperalgesic or pronociceptive activity *in vivo* (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995).

Opioid peptides have prominent neuromodulatory actions on evoked transmitter release: an opioid receptor-mediated inhibition of tachykinin release from either central or peripheral endings of capsaicin-sensitive primary afferent neurones has been repeatedly demonstrated (Maggi, 1991, for review).

In this study we aimed to ascertain whether synthetic nociceptin (orphanin FQ) has any neuromodulatory action on tachykinin release from peripheral endings of capsaicin-sensitive primary afferent neurones. With this aim we studied its effect on the contractile responses produced by electrical field stimulation (EFS) in the guinea-pig isolated renal pelvis which, as demonstrated previously (Maggi *et al.*, 1992), are exclusively mediated by release of endogenous tachykinins from sensory nerves.

Methods Male albino guinea-pigs weighing 350-400 g were stunned and bled. The kidneys and attached renal pelvis/ureter were quickly removed and placed in a Petri dish containing oxygenated Krebs solution for dissection of the renal pelvis: experiments were performed in the presence of 10 μM indomethacin, as described previously (Maggi *et al.*, 1992). The mechanical activity of the whole renal pelvis was recorded isotonically, as described by Maggi *et al.* (1992), under a resting load of 2 mN. The experiments commenced after a 60 min equilibration period. Positive inotropic responses to electrical field stimulation (EFS) were evoked at 30 min intervals. EFS was performed by means of two wire platinum electrodes placed at the top and bottom of the organ bath, connected to a GRASS S 88 stimulator: square wave pulses of maximal voltage (pulse width 0.5 ms) were delivered at a frequency of 5 Hz for 10 s. Nociceptin was added to the bath 5 min before the next stimulus. All contractile responses were expressed as % of the maximal inotropic response to 0.1 μM neurokinin A (NKA). Antagonists were added to the bath 10 min before nociceptin administration or 15 min before the next stimulus. The drugs used were nociceptin (Tocris Cookson, Bristol, U.K.), indomethacin and naloxone (Sigma, St Louis, MO, U.S.A.) naltrindole and nor-binaltorphimine (nor-BNI, RBI, Natick, MA, U.S.A.), NKA and dermorphin (Peninsula, St Helens, U.K.). MEN 10627 or cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β-5β) was synthesized in the Chemistry Dept. of Menarini Pharmaceuticals.

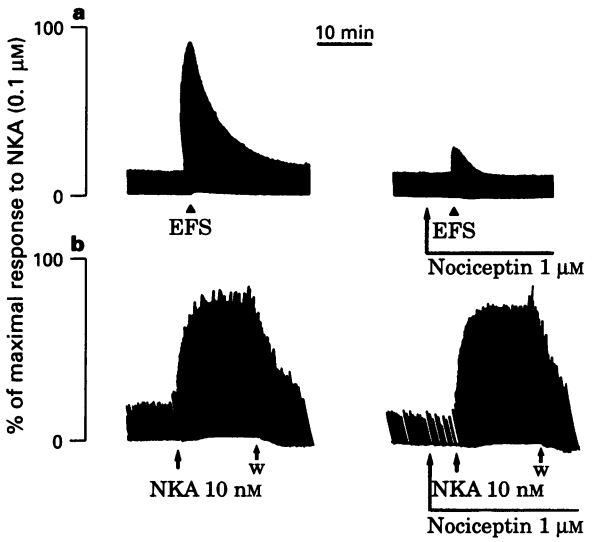

Figure 1 Tracings showing the inhibitory effect of nociceptin (1 μM) on the positive inotropic response produced by electrical field stimulation (EFS, at ▲, a) and the positive inotropic response produced by application of neurokinin A (NKA, b; W = washout) in the guinea-pig isolated renal pelvis. Note that nociceptin had no effect on spontaneous activity of the renal pelvis; nociceptin depressed the inotropic response to EFS without affecting that to NKA, indicating a prejunctional site of action of tachykinin-containing sensory nerves.

Results EFS (5 Hz for 10 s, 0.5 ms pulse width, maximal voltage) markedly increased the amplitude of spontaneous contractions of the guinea-pig isolated renal pelvis. The involvement of endogenous tachykinins was shown by the suppressant effect of the selective NK_2 receptor antagonist, MEN 10,627 (0.3 μM, $n=4$) which produced >90% inhibition of the response to EFS.

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Nociceptin (10 nM – 1 μM, 5 min beforehand) inhibited the EFS-induced inotropic response (Figure 1); the inhibitory effect of nociceptin was fully reversible on washout (not shown), thus enabling the construction of non-cumulative concentration-response curves to the neuropeptide with 30 min intervals between doses (Figure 2a). Nociceptin inhibited the inotropic response to EFS without affecting the basal spontaneous contractile activity of the renal pelvis: at 1 μM the maximal inhibition of the response to EFS was 85 ± 4% ($n = 8$); the EC_{50} was 28.4 nM (18 – 52 nM are 95% c.l.).

As shown in Figure 2b, the inhibitory effect produced by a submaximally effective concentration of nociceptin (0.3 μM) was unchanged when tested in the presence of 0.3 μM naloxone (10 min beforehand), which almost completely prevented the inhibitory action of the potent μ opioid receptor agonist, dermorphin (0.1 μM, $n = 5$, Figure 2b). The involvement of κ and δ opioid receptors in the action of nociceptin was likewise excluded: the inhibitory effect of nociceptin averaged 73 ± 3 and 67 ± 9% inhibition in the absence and presence of the κ opioid receptor antagonist, nor-BNI (1 μM, 10 min beforehand, $n = 4$); and 75 ± 4 and 78 ± 6% in the absence and presence of the δ opioid receptor antagonist, naltrindole (3 μM, 10 min beforehand, $n = 4$).

To assess whether the effect of nociceptin may have a postjunctional component, we studied its effect on the contractile response to 10 nM NKA (Figure 1) which produced a positive inotropic response averaging 73 ± 9% of the response to 0.1 μM NKA ($n = 4$); the response to 10 nM NKA was totally abolished by MEN 10,627 (0.3 μM, $n = 4$) while being unaffected by nociceptin (1 μM, + 5 ± 5% variation vs. control response, $n = 4$).

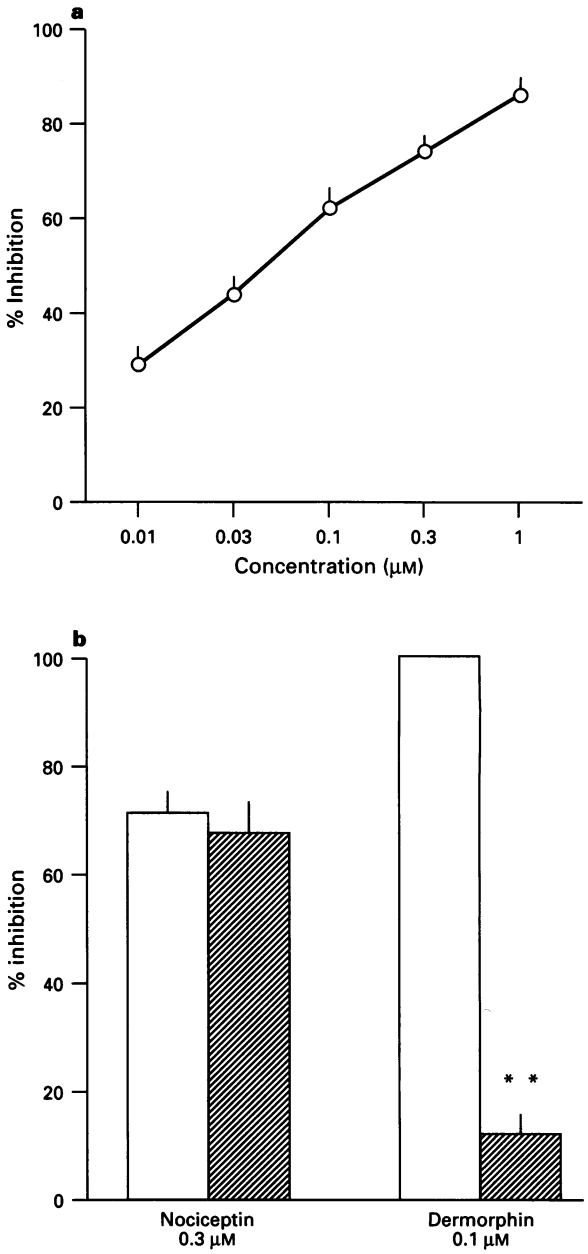

Figure 2 (a) Concentration-dependent inhibition by nociceptin of the positive inotropic response to EFS in the guinea-pig isolated renal pelvis. Each value is mean ± s.e.mean of 8 experiments. (b) Inhibition by nociceptin (0.3 μM) or dermorphin (0.1 μM) of the positive inotropic effect of EFS in the guinea-pig isolated renal pelvis in the absence (open columns) and presence (hatched columns) of naloxone (0.3 μM). Each value is mean ± s.e.mean of 5 – 8 experiments. $^{**}P < 0.01$ significantly different from the effect of dermorphin in the absence of naloxone (Student's t test for unpaired data).

Discussion The positive inotropic effect produced by EFS in the guinea-pig isolated renal pelvis is determined through the release of tachykinins from the peripheral endings of capsaicin-sensitive primary afferent neurones, predominantly acting via postjunctional NK_2 receptors (Maggi *et al.*, 1992; and present findings). Opioids have been repeatedly shown to inhibit tachykinin release from both central and peripheral endings of these sensory neurones (Maggi, 1991): this effect is thought to mediate part of their analgesic action at spinal cord level and underlies the ability of opioids to suppress neurogenic inflammation in the peripheral nervous system.

The present data indicate that nociceptin exerts a prejunctional inhibitory effect on tachykinin release from sensory nerves in the guinea-pig renal pelvis, an effect independent of the activation of either μ, δ or κ opioid receptors. The latter finding is consistent with the notion that, while sharing an opioid-like NH_2-terminal sequence, nociceptin has negligible binding affinity for μ, δ or κ opioid receptors (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). As the ORL_1 receptor is expressed in peripheral organs such as the intestine and vas deferens (Wang *et al.*, 1994), ORL_1 may mediate the prejunctional modulatory action of nociceptin unravelled in this study, although the lack of a selective antagonist for this receptor prevents a firm conclusion on this point being drawn.

Interestingly, as opioid receptors do, the ORL_1 receptor also mediates inhibition of adenylate cyclase (Mollereau *et al.*, 1994); moreover, the high degree of sequence homology in the third cytoplasmic loop of ORL_1 vs. opioid receptors suggests that ORL_1 is capable of activating the same G proteins as do opioid receptors (Mollereau *et al.*, 1994), providing a basis for the similar neuromodulatory action of nociceptin and opioid peptides on evoked release of tachykinins. According to our findings, should nociceptin be expressed/released in the periphery, a suppressant effect on neurogenic inflammation may be anticipated, as occurs with opioid peptides.

On the other hand, the present findings suggest that mechanisms other than modulation of tachykinin release should be sought to account for the pronociceptive effect of this novel neuropeptide, as they have been demonstrated in *in vivo* studies (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995).

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(Received April 1, 1996
Accepted April 22, 1996)