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European Journal of Pharmacology 388 (2000) 183–186

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Short communication

Nociceptin inhibits tonic nitric oxide release in the mouse isolated proximal colon

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Received 29 November 1999; accepted 30 November 1999

Abstract

Nociceptin/orphanin FQ, the endogenous ligand of the opioid receptor-like (ORL1) receptor, caused contractions in the isolated colon of the mouse. Tetrodotoxin and the nitric oxide (NO) synthase inhibitor *N*ω-nitro-L-arginine also produced contractions which were quantitatively similar to those seen in response to nociceptin. In the presence of either tetrodotoxin or *N*ω-nitro-L-arginine, nociceptin was unable to cause a further contraction, whereas the muscarinic receptor agonist carbachol elicited a contractile response. Nociceptin had no contractile activity in colonic preparations contracted by *N*ω-nitro-L-arginine then relaxed by addition of the NO donor sodium nitroprusside. These data suggest that nociceptin causes contractions of the mouse proximal colon by inhibiting the tonic, neuronal release of NO.

Keywords: Nociceptin; ORL1 receptor; Colon, mouse, isolated; Nitric oxide (NO)

1. Introduction

It is well-established that endogenous opioid peptides can modulate gastrointestinal motility by acting on three well-defined or ''classical'' opioid receptors, μ, δ and κ (see Kromer, 1988). More recently, the opioid receptor-like (ORL1) receptor (Mollereau et al., 1994) and its endogenous ligand, nociceptin/orphanin FQ (Meunier et al., 1995; Reinscheid et al., 1995) have been identified. Nociceptin has very low affinity for μ-, δ- and κ-opioid receptors and its actions on the ORL1 receptor are not antagonised by the prototypical opioid antagonist naloxone (Meunier et al., 1995; Reinscheid et al., 1995). Transcripts of the ORL1 receptor have been located in the gastrointestinal tract of the rat (Wang et al., 1994), as has nociceptin-like immunoreactivity (Yazdani et al., 1999). We and others have shown that nociceptin causes contractions in the isolated colon of the mouse (Corbett et al., 1998; Osinski et al., 1999; Paterson et al., 1999) and the rat (Corbett et al., 1998; Taniguchi et al., 1998; Paterson et al., 1999; Yazdani et al., 1999), which are unaffected by naloxone and result from actions at ORL1 receptors. The mechanism(s) by which nociceptin elicits colonic contractions is unclear however, and may vary between species. The aim of this study was to determine the mechanism by which nociceptin causes smooth muscle contractions in the mouse proximal colon.

2. Methods

2.1. Mouse isolated colon bioassays

Male, adult mice (strain DBA/2, weighing 25–30 g) were killed by cervical dislocation. Sections (1.5 cm) of proximal colon were removed from the junction of the colon and caecum, flushed of their contents and trimmed of mesentery. Preparations were mounted vertically under 1 g tension with fine cotton thread in 3 ml siliconised organ baths containing Krebs' solution maintained at 37°C and bubbled with 95% O_2 and 5% CO_2 then allowed to equilibrate for 60 min prior to drug addition. Tissue viability was established using carbachol (1 nM–1 μM). Isometric responses were recorded using Grass FT03C force–displacement transducers linked to a Grass four-channel pen recorder. The agonist potencies of drugs are expressed as EC_{50} values (nM): the concentration of agonist, estimated

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00858-4

from log concentration–response curves, that caused 50% of the maximal response (E_{max}).

2.2. Drugs

The composition of Krebs' solution was as follows (mM): NaCl 118, KCl 4.74, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4 \cdot 7H_2O$ 1.20, $NaHCO_3$ 25, glucose 11. Drugs used were carbachol (carbamylcholine chloride), atropine sulphate, tetrodotoxin, *N*ω-nitro-L-arginine, *N*ω-nitro-L-arginine methyl ester, L-arginine, D-arginine, sodium nitroprusside (all Sigma), nociceptin (Bachem) and naloxone (Endo Laboratories). Stock solutions of peptides were dissolved in methanol: 0.01 M CH_3COOH (50:50, v:v) containing 1 mg/ml bovine serum albumin; other drugs were made up in distilled water. All drugs were stored at −20°C and fresh dilutions made daily in Krebs' solution. Drugs were added to the organ bath in a noncumulative manner. Blocking drugs were in contact with the tissue for 3–5 min prior to addition of nociceptin.

2.3. Statistical analysis

Data are given as means ± S.E.M. or mean with range. Student's unpaired *t*-test was used to establish statistical significance; a probability level of $p < 0.05$ was considered to be significant.

3. Results

3.1. Contractile activity of nociceptin and carbachol

Nociceptin caused concentration-dependent contractions in the mouse proximal colon with an EC_{50} value of 1.3 nM (range 0.4–2.7 nM) and an E_{max} of 0.93 ± 0.06 g ($n = 6$; Fig. 1). The contractile responses were multiphasic, consisting of a rapid, poorly maintained contraction followed by slowly fading rhythmic activity (Fig. 2). The contractions were unaffected by the addition of either 300 nM naloxone ($n = 3$) or atropine (300 nM; $n = 5$).

Carbachol caused concentration-dependent contractions (EC_{50} = 385 nM, range 52–1100 nM, $E_{max} = 1.18 \pm 0.06$ g, $n = 10$; Fig. 1) which were rapid in onset and well-maintained (Fig. 2). In five preparations, the responses to submaximal concentrations of carbachol were antagonised fully by atropine but unaffected by naloxone (data not shown).

3.2. Effects of tetrodotoxin

Tetrodotoxin (0.3–1 μM) caused contractile responses which were qualitatively and quantitatively similar to those evoked by nociceptin ($E_{max} = 1.07 \pm 0.10$, $n = 5$; Figs. 1 and 2). In the presence of 1 μM tetrodotoxin, submaximal concentrations of nociceptin (300 nM) failed to elicit any additional contraction (Fig. 2). In contrast, the addition of carbachol (1 μM) in the presence of 1 μM tetrodotoxin did cause further contraction; the combined response was 1.31 ± 0.09 g ($n = 3$) which was not significantly different to that seen in response to carbachol alone ($p = 0.28$).

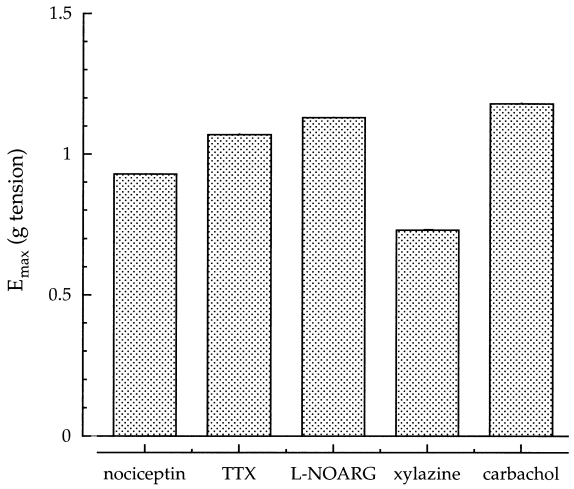

Fig. 1. Maximum contractile responses (E_{max} values) of nociceptin, tetrodotoxin (TTX), *N*ω-nitro-L-arginine (L-NOARG), xylazine and carbachol in the mouse isolated proximal colon. Each point is the mean ± S.E.M. of 4–10 observations.

3.3. Effects of inhibition of NO synthase

The nitric oxide (NO) synthase inhibitor *N*ω-nitro-L-arginine (10–30 μM) also caused contractions in colonic preparations. Although variable, these responses were concentration-dependent, relatively slow in onset and well-maintained (Fig. 2); the E_{max} was 1.13 ± 0.11 g ($n = 5$) which was not significantly different to that seen in response to either nociceptin or tetrodotoxin ($p = 0.26$; Fig. 1). The contractions to *N*ω-nitro-L-arginine were readily reversible using 100 μM L-arginine (Fig. 2) but not D-arginine. There was no additional contraction when tetrodotoxin was added to preparations already contracted by *N*ω-nitro-L-arginine ($n = 4$). Similarly, *N*ω-nitro-L-arginine had no contractile activity in colonic preparations already contracted by tetrodotoxin (10 μM; $n = 4$).

In the presence of 10 μM *N*ω-nitro-L-arginine, nociceptin (300 nM) failed to elicit any further contractile response ($n = 8$; Fig. 2). In contrast, the addition of 1 μM carbachol could elicit a contraction additional to that evoked by the NO synthase inhibitor; the maximum contractile response in the presence of both drugs was 1.12 ± 0.06 g ($n = 3$) which is not significantly different to that seen in response to carbachol alone ($p = 0.56$). Similar results were obtained with *N*ω-nitro-L-arginine methyl ester, another NO synthase inhibitor ($n = 4$).

Preparations contracted with 10 μM *N*ω-nitro-L-arginine could be relaxed using the NO donor sodium nitroprusside (Fig. 2). In the presence of both *N*ω-nitro-L-

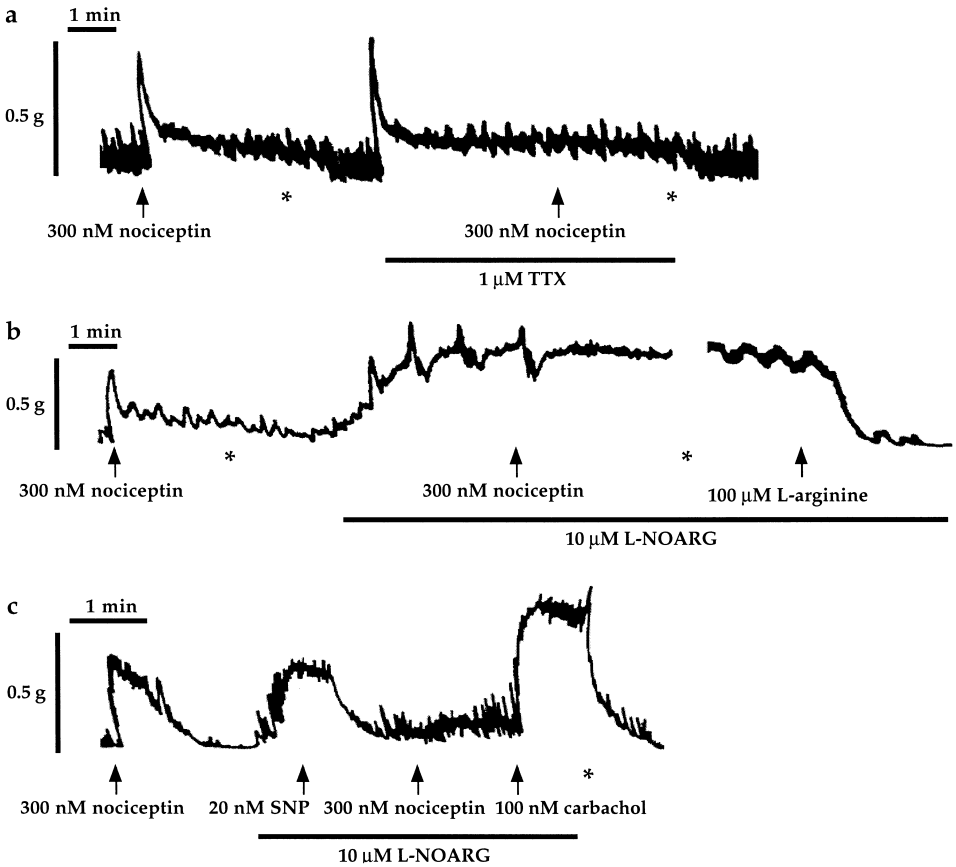

Fig. 2. Contractile effects of various drugs in the mouse isolated proximal colon. (a) Nociceptin alone and in the presence of tetrodotoxin (TTX). (b) Nociceptin alone and in the presence of *N*ω-nitro-L-arginine (L-NOARG). (c) Nociceptin alone and in preparations contracted by *N*ω-nitro-L-arginine then relaxed by the addition of sodium nitroprusside (SNP). Drugs were added at arrows and washed at ∗. Bar denotes the presence of either tetrodotoxin or *N*ω-nitro-L-arginine.

arginine and sodium nitroprusside, 300 nM nociceptin did not elicit a contractile response ($n = 4$; Fig. 2) whereas the addition of 300 nM carbachol did cause a contraction which was not significantly different to control values ($p = 0.34$, $n = 6$).

3.4. The effects of xylazine

The α_2-adrenoceptor-selective agonist xylazine caused concentration-dependent contractions of the colon (EC_{50} = 48 nM, range 27–62 nM; $E_{max} = 0.73 \pm 0.12$ g, $n = 4$; Fig. 1). Xylazine caused no additional contraction in the presence of *N*ω-nitro-L-arginine ($n = 3$). Furthermore, in preparations responding maximally to xylazine, the addition of nociceptin elicited no further contractile response.

4. Discussion

The activation of ORL1 receptors by nociceptin could cause contraction of the murine colon in three ways: (i) by promoting the release of a contractile neurotransmitter; (ii) by preventing the tonic release of an inhibitory transmitter; or (iii) by a direct action on smooth muscle. Acetylcholine is one of the most abundant excitatory neurotransmitters in the gastrointestinal tract but does not underlie the contractile effects of nociceptin as the activity of the peptide was unaffected by the muscarinic receptor antagonist atropine. To determine either if other excitatory mediators are involved or if nociceptin blocks the release of an inhibitory substance, the effects of tetrotodotoxin on nociceptin-induced contractions were examined. The addition of tetrodotoxin alone caused contractions which would strongly suggest that in the mouse proximal colon there is tonic, neuronal release of an inhibitory substance. In the presence of tetrodotoxin, nociceptin was unable to cause any additional contraction implying that the contractile activity of nociceptin likewise resulted from inhibition of release of this inhibitory transmitter.

Osinski et al. (1999) also investigated the contractile properties of nociceptin in the mouse isolated colon and suggested that they were the result of inhibition of an unidentified inhibitory myenteric mediator. Yazdani et al. (1999) drew a similar conclusion from their work on rat proximal colon in which contractions to nociceptin were abolished by tetrodotoxin but were not caused by inhibi-

tion of the release of either NO or vasoactive intestinal polypeptide; they did not discount the involvement of ATP. In contrast to Yazdani et al. (1999), Taniguchi et al (1998) discounted the possibility that the contractions in rat colon were as the result of nociceptin acting prejunctionally to inhibit the tonic release of an inhibitory substance and we showed previously that in rat distal colon the contractile effects of nociceptin are unaffected by tetrodotoxin (Menzies et al., 1999). It seems clear, therefore, that the mechanisms underlying colonic contractions to nociceptin are both species-and region-specific.

In the mouse isolated proximal colon, the most likely candidates for the tonically released inhibitory transmitter are NO, vasoactive intestinal polypeptide and ATP. The contribution of the NO system was examined using the specific NO synthase inhibitor *N*ω-nitro-L-arginine. *N*ω-nitro-L-arginine also caused contractions which were quantitatively similar to those seen in response to nociceptin and tetrodotoxin. The failure of the tissue to respond to nociceptin in the presence of the blocking drugs was not because the tissue was maximally contracted because carbachol could still elicit a contraction in the presence of these agents. When NO synthesis was inhibited with *N*ω-nitro-L-arginine then the tissue was relaxed using the NO donor sodium nitroprusside, nociceptin was still unable to cause a contraction while carbachol, presumably acting directly on smooth muscle, elicited a contraction not significantly different from controls. Thus, it appears that ORL1 receptor activation inhibits tonic neuronal NO release in the mouse proximal colon. The release of NO in this tissue is not controlled exclusively by prejunctional ORL1 receptors, however, as α_2-adrenoceptors seem to subserve a similar regulatory role since the actions of nociceptin are similar to those xylazine.

The reasons why the contractile reponses to L-NOARG are longer-lasting than those to nociceptin and tetrodotoxin are unclear but possibly may be explained by their mode of action. Tetrodotoxin (via blockade of voltage-gated Na^+ channels) and nociceptin (via ORL1-receptor activation) prevent NO release from neurons, whereas L-NOARG is known to act intracellularly to inhibit NO production.

In conclusion, the results from this investigation show that nociception activates ORL1 receptors located prejunctionally on enteric neurones of the mouse isolated proximal colon leading to an inhibition of tonic NO release resulting in a contractile response. The physiological role of the nociceptin–ORL1 system in the colon is not clear but it may be significant to pathophysiological processes that underlie motor dysfunction of the bowel.

Acknowledgements

J.R.W. Menzies was the recipient of a research studentship from Glasgow Caledonian University.

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