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In vitro agonist effects of nociceptin and [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in the mouse and rat colon and the mouse vas deferens

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Abstract

Nociceptin is an endogenous ligand of the opioid receptor-like (ORL1) receptor, a G-protein coupled receptor with sequence similarities to the opioid receptors. ORL1 receptors are present at both central and peripheral sites in several mammalian species but their functions are as yet poorly understood. The main aim of this investigation was to study the effects of nociceptin and the putative ORL1 receptor antagonist [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in two peripheral tissues, the isolated proximal colon of the mouse and the distal colon of the rat. Nociceptin, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO; μ-opioid receptor selective) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE; δ-opioid receptor selective) caused concentration-dependent contractions of mouse and rat isolated colon preparations (nociceptin EC_{50} = 1.20 and 0.28 nM in the mouse and rat, respectively). Des[Phe^1]nociceptin (250 nM) had no contractile effect. Naloxone (300 nM) antagonised the effects of DAMGO and DPDPE but had no effect in either preparation on contractions seen in response to nociceptin. [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ also caused contractions in the colonic preparations (EC_{50} = 6.0 and 3.1 nM in the mouse and rat, respectively); there was no evidence of any antagonist activity. Tetrodotoxin (1 μM) abolished the contractile effects of nociceptin in the mouse colon but had no effect in the rat. In the vas deferens preparation isolated from DBA/2 mice, nociceptin caused concentration-dependent inhibitions of electrically-evoked contractions which were antagonised by [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ (apparent pK_B = 6.31). However, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ (0.3–10 μM) also possessed agonist activity in this preparation, as it inhibited the electrically-evoked contractions in a concentration-dependent manner. These observations do not support the proposal that [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ has agonist activity at central ORL1 receptors but is an antagonist in the periphery and that these differences in efficacy point to differences in the receptors. Rather, these data along with those of others suggest that [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ is a partial agonist and that differences in receptor reserve can account for the varied pharmacological actions of this pseudopeptide at central and peripheral sites.

Keywords: Nociceptin; [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$; ORL1 receptor; Vas deferens, Mouse; Colon, Mouse; Colon, Rat

1. Introduction

Nociceptin (also named orphanin FQ) is an endogenous heptadecapeptide with sequence similarities to the endogenous opioid peptide dynorphin A (Meunier et al., 1995; Reinscheid et al., 1995). Despite this structural homology, nociceptin does not bind with high affinity to any of the ''classical'' μ-, δ- or κ-opioid receptors; instead, nociceptin is considered to be the endogenous ligand for the opioid receptor-like (ORL1) receptor (Mollereau et al., 1994). Endogenous opioid peptides and synthetic opioid receptor ligands have little affinity for ORL1 receptors and the actions of nociceptin via these receptors are not antagonised by the opioid receptor antagonist naloxone; an accepted definition of opioid activity. This not withstanding, the ORL1 receptor has sequence homology with μ-, δ- and κ-opioid receptors and on structural grounds may be classed as an opioid receptor (Henderson and McKnight, 1997).

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Nociceptin and ORL1 receptors are found in many parts of the central and peripheral nervous systems where they are likely to subserve important physiological functions (for reviews, see Henderson and McKnight, 1997; Meunier, 1997; Darland et al., 1998). What these functions are, however, remains unclear since different groups have often reported contradictory results. In particular, the effects of nociceptin on nociception are rather ambiguous since the peptide has been variously reported to cause nociception (Meunier et al., 1995; Reinscheid et al., 1995), allodynia (Okuda-Ashitaka et al., 1996) and hyperalgesia (Rossi et al., 1996) in mice. Clarification of these effects has been hampered by the lack of a selective antagonist for the ORL1 receptor. Recently, however, Guerrini et al. (1998) reported that a truncated pseudopeptide analogue of nociceptin, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$, possessed selective and fairly potent antagonist activity at the ORL1 receptor. The synthetic peptide shifted the concentration–response curves for nociceptin to the right in a parallel fashion in both the guinea-pig ileum and mouse isolated vas deferens preparations ($pA_2 = 7.20$ and 6.75, respectively). More recently, however, two groups have described agonist rather than antagonist effects of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ at central ORL1 receptors in vivo (Calo et al., 1998; Carpenter and Dickenson, 1998) leading to the proposal that two distinct populations of ORL1 receptors are present; a peripheral population, where [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ acts as an antagonist, and a central population, where [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ acts as an agonist (Calo et al., 1998).

Endogenous opioid peptides acting on μ-, δ- and κ-opioid receptors are known to be involved in the control of gastrointestinal motility in many mammalian species including man (Kromer, 1988). ORL1 receptor mRNA expression (Wang et al., 1994) and nociceptin-like immunoreactivity (Yazdani et al., 1997, 1999) have been detected in the rat gastrointestinal tract indicating that this peptide may also play a part in the control of normal gastrointestinal physiology. In support of this role, we have previously reported that nociceptin causes contractions in the isolated colon of the mouse and of the rat, presumably through ORL1 receptors (Corbett et al., 1998; Paterson et al., 1999). Taniguchi et al. (1998) and Yazdani et al. (1999) have also demonstrated the contractile activity of nociceptin in the rat colon both in vivo and in vitro, and Osinski et al. (1999) have shown nociceptin to cause contractions of murine isolated colon. In this investigation, we have used the putative ORL1 receptor antagonist [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ to confirm and extend our earlier observations. Furthermore, in the light of reports describing the central agonist effects of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ and also observations reported here of agonist activity with this pseudopeptide in the mouse and rat colon, we also examined the actions of nociceptin and [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in the isolated vasa deferentia of DBA/2 mice.

2. Materials and methods

2.1. Mouse and rat isolated colon and mouse isolated vas deferens bioassays

Male, adult mice (strain DBA/2, 25–30 g) were killed by cervical dislocation. 1.5 cm sections of proximal colon were removed in a distal direction from the junction of the colon and caecum, flushed of their contents and trimmed of mesentery. The vasa deferentia were also removed from some of these animals and vascular and fatty tissue removed. Male Wistar rats (200–250 g) were killed by cervical dislocation and exsanguination and segments of distal colon (1–2 cm) were removed from the point where the colon emerges from under the pelvic girdle. Preparations were mounted vertically under 1 g tension with fine cotton thread in 3 ml (mouse) or 20 ml (rat) siliconised organ baths containing Krebs solution maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Preparations of colon were allowed to equilibrate for 60 min and vasa deferentia for 10–15 min prior to drug addition. In preparations of isolated colon and the mouse vas deferens, drugs were added non-cumulatively and cumulatively, respectively.

Neurogenic twitch contractions were induced electrically in the mouse vas deferens using a Grass S88 stimulator via platinum electrodes at the top and bottom of the organ baths. Preparations were stimulated with trains of three square pulses delivered at 5 Hz every 10 s (0.5 ms pulse duration). Sensitivity was established using [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO; data not shown) in the vas deferens and carbachol (carbamylcholine chloride) in the mouse and rat colon. Isometric responses were recorded using Grass FT03C force displacement transducers linked to a Grass four channel pen recorder. Agonist activities are expressed as EC_{50} or IC_{50} (nM): the concentration of agonist, estimated from log concentration–response curves, that caused 50% of the maximal response (E_{max}). Data are expressed as mean ± S.E.M. or mean and range. Since [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ was found to act as a partial agonist in the mouse isolated vas deferens, the ability of the pseudopeptide to antagonise the effects of nociceptin was assessed using the method of Kosterlitz and Watt (1968).

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, $MgSO_4$, $7H_2O$ 1.20, $NaHCO_3$ 25, glucose 11; in experiments using the mouse vas deferens Mg^{2+}-free Krebs solution was used (Hughes et al., 1975). Drugs used

were carbamylcholine chloride (carbachol), tetrodotoxin, DAMGO, [D-Pen^2, D-Pen^5]enkephalin (DPDPE, all Sigma), U69,593 ([5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzene acetamide, Upjohn), CI 977 ((5*R*)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrodinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide, A.T. McKnight, Parke-Davis), naloxone (Endo Laboratories), nociceptin (Bachem), [Tyr^{14}]nociceptin, des[Phe^1]nociceptin (both Phoenix Pharmaceuticals) and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ (Tocris Cookson).

Stock solutions of peptides were dissolved in methanol: 0.01 M CH_3COOH (50:50, v:v) containing 1 mg/ml bovine serum albumin to reduce adsorptive losses; other drugs were made up in distilled water. All drugs were stored at −20°C and fresh dilutions made daily in Krebs solution.

3. Results

3.1. Contractile activity of nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in the mouse isolated proximal colon and rat isolated distal colon

In the mouse isolated proximal colon, contractile responses to nociceptin consisted of a rapid, transient ''twitch'' contraction followed by slowly fading rhythmic activity (Fig. 1a). These phasic contractions were concentration-dependent with an EC_{50} value of 1.2 nM (range 0.7–2.7 nM, $E_{max} = 0.94 \pm 0.09$ g, $n = 4$; Fig. 2a). Des[Phe^1]nociceptin had no effect ($n = 4$).

It was not possible to test if the agonist activity of nociceptin was antagonised by the putative ORL1 antagonist [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ as this pseudopeptide also caused concentration-dependent phasic contractions in the mouse proximal colon ($EC_{50} = 6.0$ nM, range 5.3–8.0 nM, $E_{max} = 0.73 \pm 0.08$ g, $n = 4$; Figs. 1a and 2a). The contractile responses of both nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ were unaffected by 300 nM naloxone ($n = 4$; Fig. 1b). Tetrodotoxin (1 μM) abolished the contractile effects of both ORL1 receptor ligands ($n = 6$).

In the rat distal colon, nociceptin and a second ORL1 receptor agonist, [Tyr^{14}]nociceptin (Nicholson et al., 1998) caused concentration-dependent contractions with EC_{50} values of 0.28 nM (range 0.14–0.48 nM, $n = 8$) and 0.62 nM (range 0.12–1.13 nM, $n = 5$), respectively (Fig. 3a). These responses were unaffected by 300 nM naloxone. The contractile responses in the rat were less well-maintained than those in the mouse colon; the initial rapid contraction faded quickly and less tonic rhythmic contractile activity was seen. Des[Phe^1]nociceptin was without contractile activity at concentrations up to 250 nM. [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ was also an agonist with an EC_{50} value of 3.1 nM (range 1.1–5.6 nM, $n = 6$; Fig. 3a). At low concentrations (10–100 nM), there was no evidence of [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ having any antagonist activity in either the mouse or rat isolated colon. Tetrotodotoxin (1 μM) had no effect on the contractile response of 10–300 nM nociceptin ($n = 6$).

In both preparations, the agonist responses to nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ (300 nM) were unchanged in the presence of 300 nM atropine (data not shown). In the mouse isolated colon, carbachol caused concentration-dependent contractions ($EC_{50} = 615$

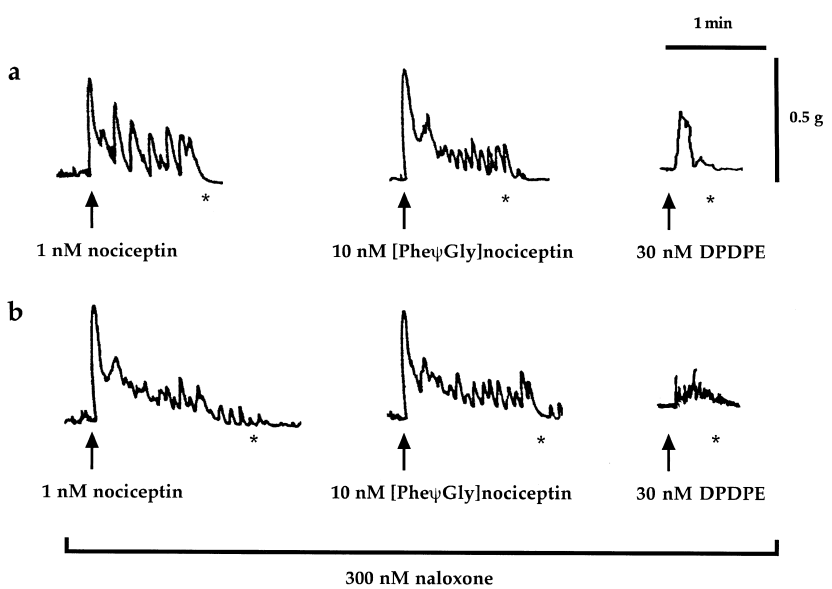

Fig. 1. (a) Contractile effects of nociceptin (1 nM), [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ ([PheψGly]nociceptin; 10 nM) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE; 30 nM) in the mouse isolated proximal colon. (b) Effect of 300 nM naloxone on the contractile response of nociceptin (1 nM), [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ ([PheψGly]nociceptin; 10 nM) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE; 30 nM) in the mouse isolated proximal colon. Drugs were added at arrows and washed at *. Bar denotes the presence of 300 nM naloxone.

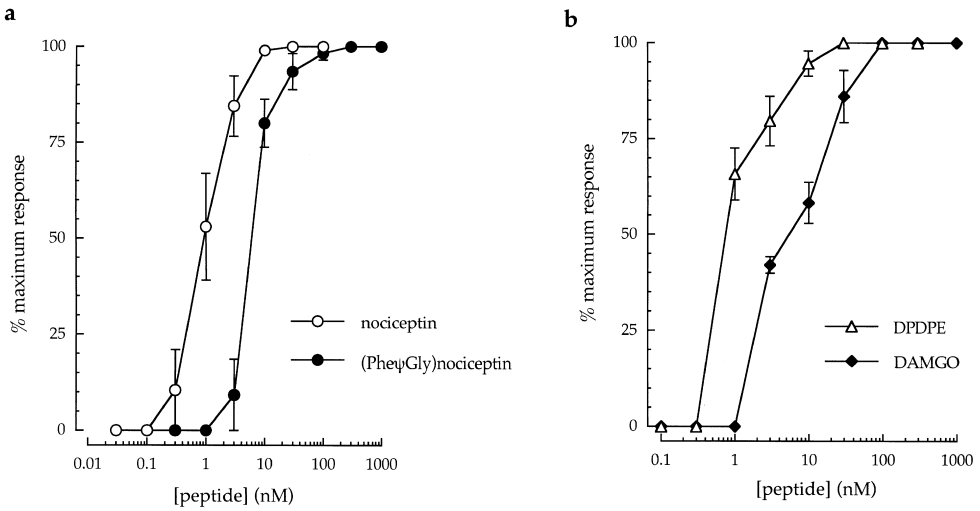

Fig. 2. (a) Effects of nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ ([PheψGly]nociceptin) in the mouse isolated proximal colon. (b) Effects of [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) in the mouse isolated proximal colon. Each point is the mean ± S.E.M. of four to five observations.

nM, range 250–1100 nM, $E_{max} = 1.51 \pm 0.21$ g, $n = 5$) which were antagonised by 100 nM atropine ($n = 3$). The contractile response to carbachol was rapid in onset and well-maintained.

3.2. Contractile activity of opioid receptor selective ligands in the mouse isolated proximal colon and rat isolated distal colon

In the mouse proximal colon, DAMGO (μ-opioid receptor selective) caused concentration-dependent contractions with an EC_{50} value of 6.4 nM (range 3.2–13.0 nM, $E_{max} = 0.94 \pm 0.09$ g, $n = 4$; Fig. 2b). DPDPE (δ-opioid receptor selective) also caused concentration-dependent contractions in this preparation ($EC_{50} = 0.74$ nM, range 0.6–1.0 nM, $E_{max} = 0.39 \pm 0.07$ g, $n = 5$; Fig. 2b). These contractile responses were qualitatively similar to those seen in response to nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$. In contrast to the ORL1 receptor selective peptides, the responses to μ- and δ-opioid receptor selective peptides were fully antagonised by 300 nM naloxone (Fig. 1b). The κ-opioid receptor selective agonist U69,593 elicited small (< 5% DAMGO E_{max}) contractile responses which were not antagonised by naloxone (300 nM).

In the rat colon, the selective δ-opioid receptor ligand DPDPE ($EC_{50} = 7.88$ nM, range 0.86–14.07 nM, $E_{max} = 1.86 \pm 0.17$ g, $n = 8$; Fig. 3b) and the selective μ-opioid receptor ligand DAMGO ($EC_{50} = 8.31$ nM, range 5.06–14.08 nM, $E_{max} = 2.23 \pm 0.42$ g, $n = 5$; Fig. 3b) also

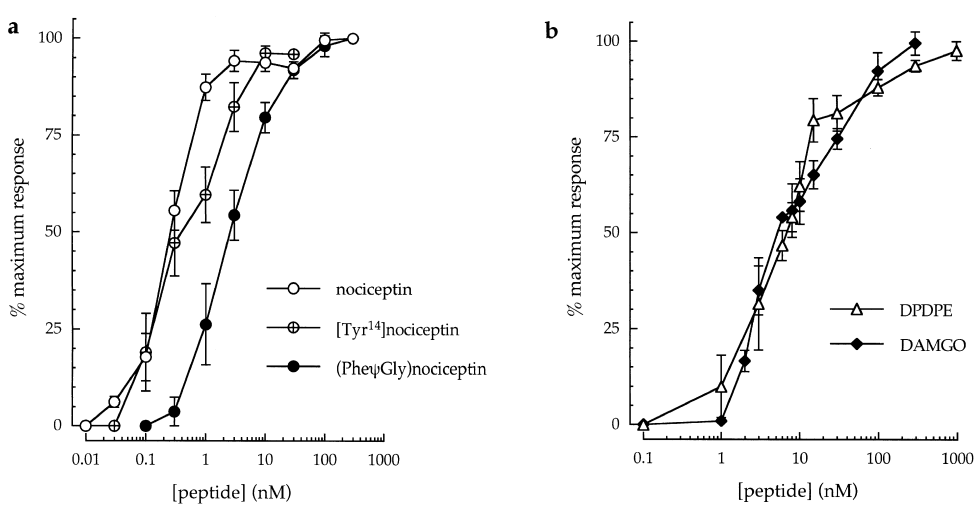

Fig. 3. (a) Effects of nociceptin, [Tyr^{14}]nociceptin and [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ ([PheψGly]nociceptin) in the rat isolated distal colon. (b) Effects of [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) in the rat isolated distal colon. Each point is the mean ± S.E.M. of six observations.

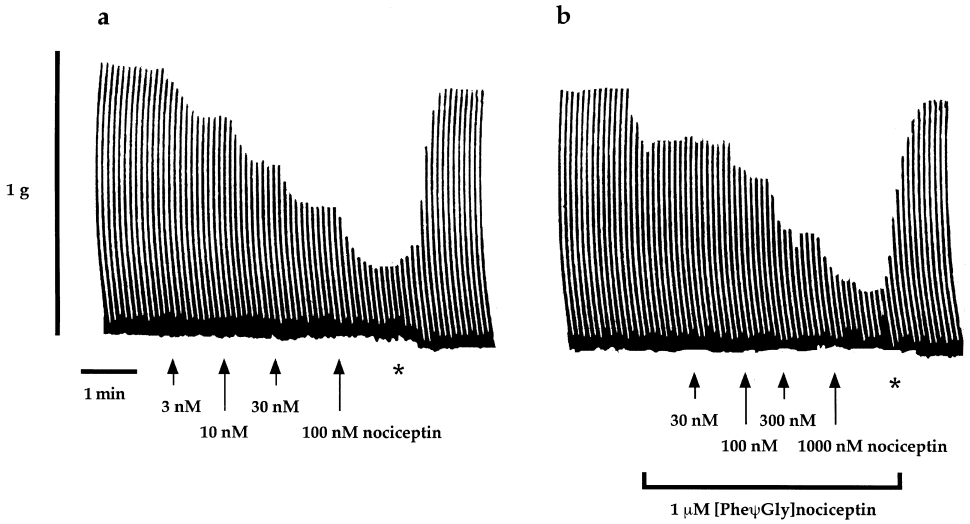

Fig. 4. Effects of nociceptin on electrically-evoked concentrations in the mouse isolated vas deferens in the absence (a) and presence (b) of 1 μM [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ ([PheψGly]nociceptin). Drugs were added at arrows and washed at *. Bar denotes the presence of 1 μM [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$.

produced concentration-dependent contractions which were antagonised by naloxone (300 nM). CI 977, a κ-opioid receptor selective ligand, had no agonist activity in 10 preparations.

3.3. Agonist and antagonist activity of [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in the mouse vas deferens

In the mouse isolated vas deferens, nociceptin inhibited the electrically-evoked contractions in a concentration-dependent and reversible fashion (IC_{50} = 51.1 nM, range = 21.0–100.0 nM, n = 13). The responses were not antagonised by the non-selective opioid receptor antagonist naloxone (300 nM, n = 6). Des[Phe^1]nociceptin was without activity up to a concentration of 1 μM (n = 4). High concentrations (0.3–10 μM) of [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ caused rapid, reversible and concentration-dependent inhibitions of electrically-evoked contractions (IC_{50} = 4.43 μM, range 4.0–4.9 μM, n = 8). These responses were not antagonised by naloxone (300 nM, n = 6). As well as the overt agonist response of the pseudopeptide, [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ (300 nM) was also able to antagonise the effects of nociceptin (Fig. 4). Using the method of Kosterlitz and Watt (1968), the apparent K_B for [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ was 479 ± 68 nM (apparent pK_B = 6.31).

4. Discussion

The results from this investigation do not support the proposal (Calo et al., 1998) that [Phe^{1}ψ(CH$_2$-NH)-Gly^2]nociceptin(1–13)NH$_2$ is an antagonist at peripheral ORL1 receptors and an agonist at central ORL1 receptors. [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ caused phasic contractions in rat and mouse isolated colon which were qualitatively and quantitatively similar to those seen with nociceptin; there was no evidence of any antagonist activity in these peripheral tissues. In addition, others have shown that [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ is an antagonist at central ORL1 receptors, blocking the actions of nociceptin both in vivo (Chu et al., 1999a) and in vitro (Chu et al., 1999b). There is convincing evidence, however, that [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ can have antagonist activity at peripheral ORL1 receptors, not only from the original description of the drug in the guinea-pig small intestine and mouse vas deferens preparations (Guerrini et al., 1998), but also in the guinea-pig isolated bronchus (Rizzi et al., 1999) and in vivo where it has been shown to decrease nociceptin-induced hypotension and bradycardia (Madeddu et al., 1999).

In the light of the agonist activity of [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ in colonic preparations, the effects of this compound were examined in the mouse isolated vas deferens. In vasa deferentia from DBA/2 mice, [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ caused frank, naloxone-insensitive inhibitions of the electrically-evoked contractions, another demonstration of the efficacy of the nociceptin analogue at peripheral ORL1 receptors. Although the agonist activity of [Phe^{1}ψ(CH$_2$-NH)-Gly^2]nociceptin(1–13)NH$_2$ precluded the assessment of any antagonist actions in colonic preparations, this drug did antagonise the actions of nociceptin in the mouse isolated vas deferens with an apparent pK_B value comparable to the previously published value (6.31 cf. 6.75; Guerrini et al., 1998). Thus, [Phe^{1}ψ(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ is a partial agonist in vasa deferentia isolated from mice of the DBA/2 strain.

It is possible that Guerrini et al. (1998) did not observe agonist activity in the mouse vas deferens because of their use of different electrical stimulation parameters but it is more likely that the vasa from Swiss mice have a low receptor reserve in comparison with the DBA/2 mice used in this study. Toll et al. (1998) demonstrated that in Chinese hamster ovary cells and SH-SY5Y cells transfected with human ORL1 receptors, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ could be either a partial agonist or antagonist depending on receptor number; the pseudopeptide was a ''full antagonist'' in cells expressing low numbers of ORL1 receptor numbers. A clear partial agonist profile for [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ was also shown in murine N1E-115 neuroblastoma cell lines endogenously expressing a low number of ORL1 receptors (Olianas et al., 1999). In contrast, Butour et al. (1998) found the nociceptin analogue to be a full agonist in Chinese hamster ovary cells expressing cloned ORL1 receptors which were presumably expressed in high numbers resulting in a large receptor reserve.

[Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ clearly has a variety of agonist and antagonist activities at ORL1 receptors in central and peripheral sites and these different pharmacological profiles can be explained by differences in the receptor reserve found in the different tissues. In isolated peripheral tissues, it is an agonist in the colon, a partial agonist in the vas deferens (from DBA/2 mice) and guinea-pig bronchi (Shah et al., 1998) and an antagonist in the guinea-pig ileum and vasa from Swiss mice. Thus, in the small intestine there would appear to be few, if any, spare ORL1 receptors whereas there is a large receptor reserve in the colon from the mouse and the rat. This is similar to the situation with morphine at μ-opioid receptors, which is a full agonist in the guinea-pig ileum but a partial agonist in the rat isolated vas deferens which has a low receptor reserve (Leslie, 1987). Differences in receptor reserve would also account for the contrasting pharmacological actions of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ at central sites. Although Mathis et al. (1997) presented evidence for more than one type of ORL1 binding site in mouse brain, binding studies in mouse forebrain (Varani et al., 1998) and rat, rabbit and guinea-pig whole brain (Nicholson et al., 1998) detected only a single ORL1 binding site. The pharmacological profile of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ reflects, therefore, a low intrinsic efficacy at ORL1 receptors.

The mechanism(s) by which nociceptin elicits contractile responses in the colon is unclear. Nociceptin is not acting indirectly via the release of acetylcholine, the major excitatory neurotransmitter in the gut, since atropine has no effect on the responses. Taniguchi et al. (1998) discounted the possibility that the contractions in rat colon were a result of nociceptin acting prejunctionally to inhibit the tonic release of an inhibitory substance. In contrast, Osinski et al. (1999) and Yazdani et al (1999) have suggested that the contractile actions of nociceptin may be the result of inhibition of an inhibitory enteric pathway. Our own preliminary data with TTX in the mouse colon, but not the rat colon, are consistent with nociceptin causing contraction by inhibiting the release of an inhibitory substance. Confirmation of these findings and identification of the inhibitory transmitter await further experimentation.

It is clear from this and other studies that nociceptin acting at ORL1 receptors has profound and varied effects on gastrointestinal motility. The physiological significance of these actions is still unclear but it is of particular interest that nociceptin causes contractions in the colon but not in either the small intestine (Nicholson et al., 1998; Yazdani et al., 1999) or the stomach of the rat (Yazdani et al., 1999). The nociceptin/ORL1 system may therefore provide a novel therapeutic target for treatment of motor dysfunction in specific regions of the bowel. The development, therefore, of a selective antagonist which is devoid of efficacy at the ORL1 receptor is, therefore, of particular importance to determine the functions of this fledgling system not only in the gut but in other systems.

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