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SPECIAL REPORT

Nociceptin inhibits cough in the guinea-pig by activation of ORL_1 receptors

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We studied the central and peripheral antitussive effect of ORL_1 receptor activation with nociceptin/orphanin FQ in conscious guinea-pigs. In guinea-pig cough studies, nociceptin/orphanin FQ (10, 30, and 90 μg) given directly into the CNS by an intracerebroventricular (i.c.v.) route inhibited cough elicited by capsaicin exposure by approximately 23, 29 and 52%, respectively. The antitussive activity of nociceptin/orphanin FQ (90 μg, i.c.v.) was blocked by the selective ORL_1 antagonist [$Phe^1\gamma(CH_2\text{-}NH)Gly^2$]nociceptin-(1-13)-$NH_2$ (180 μg, i.c.v.) and J113397 (10 mg kg^{-1}, i.p.) but not by the opioid antagonist, naltrexone (3 mg kg^{-1}, i.p.). Furthermore, intravenous (i.v.) nociceptin/orphanin FQ (1.0 and 3.0 mg kg^{-1}) also inhibited cough approximately by 25 and 42%, respectively. These findings indicate that selective ORL_1 agonists display the potential to inhibit cough by both a central and peripheral mechanism, and potentially represent a novel therapeutic approach for the treatment of cough.

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Abbreviations: CHO, Chinese hamster ovary; i.c.v., intracerebroventricular; ORL_1, 'opioid-like' orphan receptor

Introduction Nociceptin, also known as orphanin FQ, is the endogenous peptide ligand for the recently discovered 'opioid like' orphan (ORL_1) receptor reported to play an important role in pain, anxiety and appetite regulation (Meunier *et al*., 1995; Calo *et al*., 2000). The G protein coupled ORL_1 receptor, which was identified from a human cDNA library, has been found to share significant homology with classical opioid receptors ($\approx$65% in the transmembrane domains), namely, μ, κ and δ receptors (Mollereau *et al*., 1994). Notwithstanding, the pharmacology of nociceptin is distinct from opioids in that nociceptin does not have significant binding affinity for opioid receptors and opioid antagonists such as naltrexone do not block the activity of nociceptin.

ORL_1 receptors are distributed both in the CNS and in the periphery. In the CNS, activation of ORL_1 receptors by nociceptin inhibits the release of several neurotransmitters, including noradrenaline, serotonin, glutamate, dopamine and acetylcholine (Calo *et al*., 2000). In the periphery, nociceptin has also been shown to inhibit excitatory non-adrenergic non-cholinergic responses in isolated bronchi (Shah *et al*., 1998). The functional significance of this action remains to be fully elucidated. More recently however, Corboz *et al*. (2000) have shown that nociceptin inhibits capsaicin-induced bronchoconstriction in the isolated guinea-pig lung. This effect implies an inhibition of tachykinin release from C-fibres in the lung (Shah *et al*., 1998). Because capsaicin-sensitive C-fibres also play a role in the generation of cough, we sought to evaluate the activity of nociceptin on airway sensory nerve responses that contribute to cough.

Opioids are the most effective drugs available to treat cough associated with pulmonary diseases such as, upper respiratory infections, chronic bronchitis, pulmonary neoplasm and asthma (Adcock, 1991). Drugs that activate μ receptors, such as codeine and butorphanol have however significant side effect liabilities including respiratory depression, constipation and physical dependency. Thus, there is a clinical need to develop new drugs that elicit antitussive activity without the side effect liabilities of opioid agonists. To date, the potential antitussive activity of nociceptin has not been evaluated. In the present study we investigated the effect of central and peripheral ORL_1 receptor activation with nociceptin in a guinea-pig irritant-induced cough model.

Methods *Binding assay* ORL_1 and opioid receptor binding assays were performed as described by Fawzi *et al*. (1997) and Corboz *et al*. (2000). Briefly, CHO cell membranes expressing the human ORL_1 receptors were incubated with [^{125}I][Tyr^{14}]nociceptin FQ and increasing concentrations of compounds in binding assay buffer containing (mM): HEPES 50 (pH 7.4), $CaCl_2$ 2.5, $MgCl_2$ 1, NaCl 10, 0.025% bacitracin, and 0.1% bovine serum albumin. Assays were performed at room temperature for 1 h and were terminated by rapid filtration over GF/B membranes. Radioactivity retained on filters was determined in a Packard Top-Count microplate scintillation counter. K_i values were determined using curve fitting and data analysis by the program

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GraphPad Prism (GraphPad Software, San Diego, CA, U.S.A.). All assays were performed in duplicates. Total and non-specific binding was determined in quadruplicates.

Opioid receptor binding assays were performed on CHO cell membranes expressing the human opioid receptors (Receptor Biology, Beltsville, MD, U.S.A.) as described by Corboz *et al*. (2000). Briefly, CHO cell membranes were incubated with [^{3}H]-diprenorphine and increasing concentrations of compounds in binding assay buffer for 1 h at room temperature. Assays were terminated by rapid filtration over GF/B membranes and radioactivity retained on filters was counted in a Packard Top-Count. K_i values were determined using curve fitting and analysis of data by the program GraphPad Prism as described above.

Animal care and use These studies were performed in accordance to the NIH Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act in an AAALAC-accredited program.

Implantation of chronic intracerebroventricular cannula Male Hartley guinea-pigs (450–550 g, Charles River, Bloomington, MA, U.S.A.) were instrumented with a chronic intracerebroventricular (i.c.v.) guide cannula according to the methods of McLeod *et al*. (1991). Briefly, guinea-pigs were anaesthetized with a combination of ketamine (100 mg kg^{-1}, i.m) and xylazine (5 mg kg^{-1}, i.m.). Animals were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.) and a 22 gauge stainless steel guide cannula (Plastic One, Roanoke, VA, U.S.A.) was lowered into the lateral ventricle with the following coordinates relative to bregma: 0.5 mm A, 2.0 mm L and −4.5 mm V. The guide cannula was anchored to the skull by two 3.2 mm stainless steel mounting screws and dental acrylic. Drugs were administered in a 15 μl maximum volume through an injection cannula connected to Hamilton syringe with PE-20 tubing. Cough studies were conducted with these animals 1 week after i.c.v. cannula placement. For i.v. cough studies, a second group of guinea-pigs were instrumented with a jugular catheter under isoflurane (3%) anaesthesia. The i.v. studies were preformed 1 day after the jugular surgery.

Cough studies The effect of i.c.v. and i.v. nociceptin was evaluated in conscious guinea-pigs against capsaicin-induced cough following the methods of Bolser *et al*. (1993). This model is widely used to evaluate the activity of potential antitussive drugs. Overnight fasted guinea-pigs were placed in a transparent 14″×4″ Plexiglas cylinder chamber and exposed to aerosolized capsaicin (300 μM, for 4 min) produced by a Ultra-NeB 99 Devilbiss nebulizer (Somerset, PA, U.S.A.) to elicit cough. Each animal was exposed only once to capsaicin. The number of coughs were detected by a microphone placed in the chamber and verified by a trained observer. The signal from the microphone was relayed to a polygraph that provided a record of the number of coughs. To evaluate the central antitussive activity of nociceptin either physiological saline vehicle or nociceptin (10, 30, and 90 μg, i.c.v.) was given 15 min before capsaicin challenge. For antagonist studies, either the peptide nociceptin antagonist, [Phe$^1\psi$ (CH_2-NH) Gly^2] nociceptin-(1-13)-NH_2 (180 μg), the nonpeptide antagonist J113397 (10 mg kg^{-1}, i.p.) or the opioid receptor antagonist naltrexone (3 mg kg^{-1}, i.p.) was given 15 min before nociceptin (90 μg, i.c.v.). To evaluate the peripheral antitussive activity of nociceptin, animals were given either saline vehicle or nociceptin (0.3–3.0 mg kg^{-1}, i.v.) immediately before capsaicin. In a separate group of animals J113397 (10 mg kg^{-1}, i.p.) was given 15 min before nociceptin (3.0 mg kg^{-1}, i.v.).

Drugs [^{125}I][Tyr^{14}]nociceptin (2200 Ci mmol^{-1}) was obtained from Amersham-Pharmacia Biotech (Cardiff, U.K.). [^{3}H]-Diprenorphine (58 Ci mmol^{-1}) was purchased from New England Nuclear (Boston, MA, U.S.A.). Nociceptin and Phe$^1\psi$(CH_2-NH)Gly^2]-nociceptin-(1-13)-NH_2 (180 μg, i.c.v.) were purchased from Tocris (Ballwin, MO, U.S.A.). Capsaicin was purchased from Sigma Chemical Co. (St. Louis MO, U.S.A.) and J113397 (10 mg kg^{-1}, i.p.) was synthesized at Schering Plough Research Institute. Drug doses refer to their respective free bases. Capsaicin was dissolved in 10% ethanol and 90% physiological saline (0.9%). All other drugs were dissolved in physiological saline.

Statistics Data from the cough studies are expressed in total number of coughs. Values displayed in the figures represent the mean ± s.e.mean of 6–12 animals per group. Data were evaluated using a non parametric Kruskal Wallis in conjunction with a Mann Whitney *U*-test. Statistical significance was set at $P<0.05$.

Results *Binding studies* Binding studies were conducted to determine and confirm the selectivity of the agents used in the cough studies. Both nociceptin/orphanin FQ and the ORL_1 peptide receptor antagonist show a high affinity for the ORL_1 receptor and >1000 fold selectivity over opioid receptors (Table 1). The nonpeptidic ORL_1 receptor antagonist J113397 has been shown to have a low nanomolar potency over the ORL_1 receptor and a high degree of selectivity over opioid receptors (Corboz *et al*., 2000; Ozaki *et al*., 2000). In contrast, naltrexone shows a high affinity for the μ opioid receptor and >1000 fold selectivity over the ORL_1 receptor (Table 1).

Effect of nociceptin on capsaicin-induced cough In control animals, aerosolized capsaicin (300 μM) produced a mean of 13±2 coughs during a 4 min capsaicin exposure. Under the present experimental conditions, 4 min exposure to capsaicin produced maximum cough responses without observable respiratory distress. Nociceptin (10, 30, and 90 μg, i.c.v.) given 15 min before capsaicin challenge dose-dependently

Table 1 K_i of nociceptin, naltrexone and ORL_1 receptor antagonists against ORL_1 and opioid receptors

	K_i (nM)[a]			
Receptor	*Nociceptin*	*Naltrexone*	*J113397*[b]	*Peptide antagonist*
ORL_1	0.13±0.01	3818±1185	2.0±0.2	0.6±0.1
δ-opioid	No effect[c]	28.7±3.4	46,208±3845	No effect[c]
κ-opioid	>10,000	2.9±0.1	835±162	8732±1439
μ-opioid	3030±371	0.3±0.02	177±4	4174±524

[a]K_i values are mean±s.e.mean ($n=4$). [b]K_i values for J113397 were reported by Corboz *et al*. (2000). [c]No effect at concentration up to 10 μM.

inhibited cough responses by approximately 23, 29 and 52%, respectively (Figure 1). No overt behavioural effects were observed after central nociceptin treatment during the course of the experiment. Figure 2 shows that the antitussive effect of nociceptin (90 μg, i.c.v.) was significantly blocked by the pretreatment with i.c.v. peptide nociceptin antagonist, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ (180 μg) and the nonpeptide antagonist J113397 (10 mg kg^{-1}, i.p.). In contrast, Figure 2 also shows that the opioid receptor antagonist naltrexone (3 mg kg^{-1}, i.p.), did not block the antitussive effects of nociceptin (90 μg, i.c.v.). Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ (180 μg, i.c.v.) and J113397 (10 mg kg^{-1}, i.p.) given alone did not significantly affect the cough responses to capsaicin (Figure 3A). The dose of naltrexone (3 mg kg^{-1}, i.p.) used in the current study was found to block the antitussive effect of morphine (60 mg kg^{-1}, i.p.) by 97% (Figure 3B). In separate studies, intravenous nociceptin (0.3–3 mg kg^{-1}) administered before exposure to capsaicin dose dependently inhibited cough (Figure 4). J113397 (10 mg kg^{-1}, i.p.), blocked the antitussive action of i.v. nociceptin (3 mg kg^{-1}).

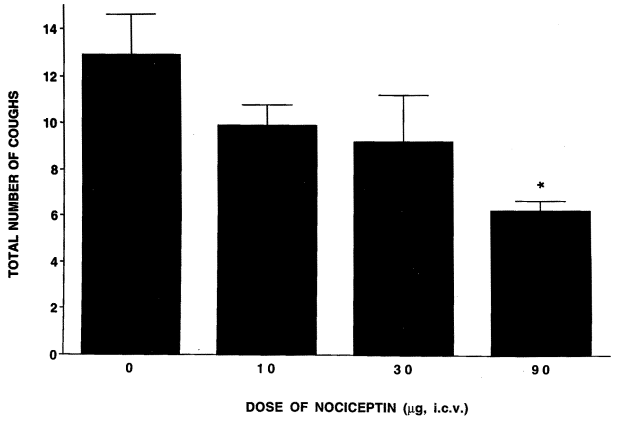

Figure 1 Antitussive effect of centrally administered nociceptin (10, 30, 90 μg, i.c.v) in the guinea-pig. Each bar represents the mean$\pm$ s.e.mean. (*$P<0.05$ compared to control animals).

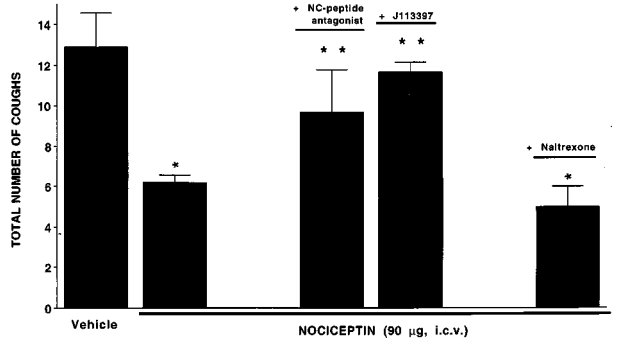

Figure 2 Effect of ORL$_1$ blockade on the antitussive effect of centrally administered nociceptin. Figure illustrates the antitussive effect of nociceptin (90 μg, i.c.v.) administered alone and in guinea-pigs treated with either Phe$^1\psi$(CH$_2$-NH)Gly^2]-nociceptin-(1-13)-NH (NC-peptide antagonist; 180 μg, i.c.v.), J113397 (10 mg kg^{-1}, i.p.) or naltrexone (3 mg kg^{-1}, i.p.). Each bar represents the mean$\pm$ s.e.mean. (*$P<0.05$ compared to control animals; **$P<0.05$ compared to guinea-pigs given nociceptin (90 μg, i.c.v.).

Discussion This is the first study demonstrating that nociceptin given by a central and peripheral route inhibits

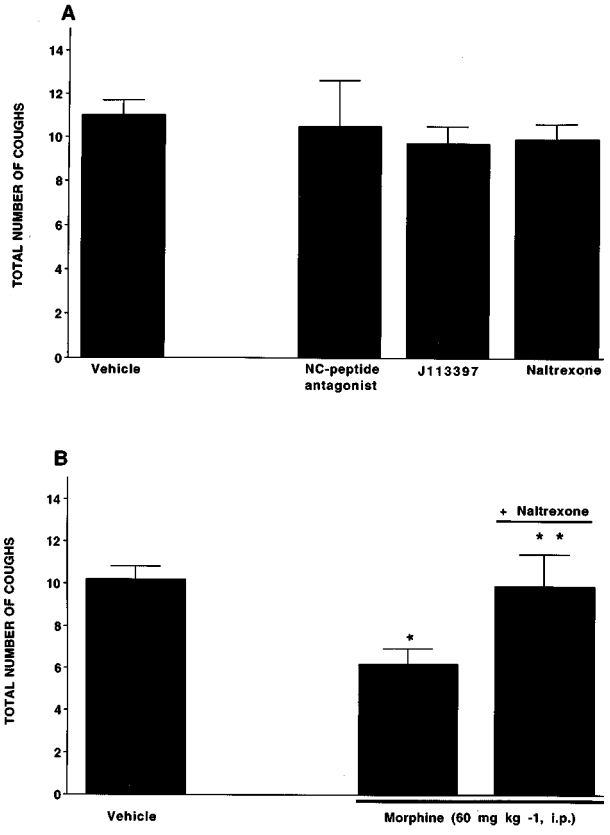

Figure 3 (A) The effect of Phe$^1\psi$(CH$_2$-NH)Gly^2]-nociceptin-(1-13)-NH (NC-peptide antagonist; 180 μg, i.c.v.), J113397 (10 mg kg^{-1}, i.p.) and naltrexone (3 mg kg^{-1}, i.p.) on capsaicin-induced cough. (B) The effect of naltrexone (3 mg kg^{-1}, i.p.) on the antitussive actions of morphine (60 mg kg^{-1}, i.p.). Each bar represents the mean$\pm$ s.e.mean. (*$P<0.05$ compared to control animals; **$P<0.05$ compared to guinea-pigs given morphine (60 mg kg^{-1}, i.p.).

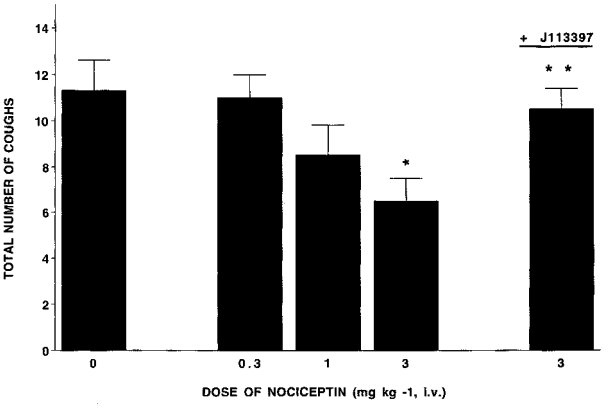

Figure 4 Antitussive effect of intravenously administered nociceptin in the guinea-pig. Figure illustrates the dose-dependent antitussive effect of nociceptin (0.3, 1.0 and 3.0 mg kg^{-1}, i.v.). Figure also illustrates the effect of J113397 (10 mg kg^{-1}, i.p.) on the antitussive effect of nociceptin (3 mg kg^{-1}, i.v.). Each bar represents the mean$\pm$s.e.mean. (*$P<0.05$ compared to control animals; **$P<0.05$ compared to guinea-pigs given nociceptin (3 mg kg^{-1}, i.c.v.).

chemical-induced cough. Because it is unlikely that the peptide nociceptin crosses the blood brain barrier to a significant extent when given i.v. or i.c.v., our study indicates that an ORL_1 agonist may exert an antitussive effect by acting at either site. The present results also show that the antitussive efficacy of nociceptin is equivalent when administered centrally or peripherally. Based on these findings, it is suggested that an nonpeptide ORL_1 agonist that crosses the blood brain barrier may activate both central and peripheral ORL_1 receptors to produce antitussive activity of greater efficacy than what is achieved by sole activation of either site. Nevertheless, the relative contribution of central and peripheral ORL_1 receptors to the regulation of responses to tussigenic stimuli requires further investigation.

The evidence that the antitussive effect of nociceptin is mediated by ORL_1 receptors is supported by the following observations. First, nociceptin selectively binds ORL_1 receptors over classical opioid receptors. We confirm earlier reports that demonstrated that nociceptin does not significantly bind μ, κ or δ receptors (Reinscheid *et al*., 1996). Secondly, we show that the ORL_1 antagonists, Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH and J113397 blocked the antitussive activity of nociceptin. The functional antagonist activity of these drugs at ORL_1 receptors has been previously described (Bigoni *et al*., 2000). Furthermore, we also show that, Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH and J113397 preferentially bind ORL_1 receptors compared to μ, κ and δ receptors. Finally, we demonstrate that the antitussive activity of nociceptin is naltrexone insensitive. This confirms that classical opioid receptors are most likely not involved in the cough suppressant activity of nociceptin. In conclusion, we propose that activation of ORL_1 receptors with selective ORL_1 agonists may be a novel approach for the treatment of cough. ORL_1 agonists may have important advantages over opioid antitussive agents because they may be devoid of μ receptor-mediated side effects such as sedation, respiratory depression, constipation or addiction liability.

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