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# Antitussive action of nociceptin in the cat

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**Abstract**

Experiments were conducted to determine the influence of the specific ORL1 receptor agonist, nociceptin, on the cough reflex in the cat. Cats were anesthetized and allowed to breathe spontaneously. Cough was elicited by mechanical stimulation of the intrathoracic airway. Intravenous administration of nociceptin (0.001–3.0 mg $kg^{-1}$) inhibited cough number and the magnitude of abdominal muscle electromyogram (EMG) discharge during cough in a dose-dependent manner. Nociceptin had no effect on the magnitude of the inspiratory muscle EMG during cough. These effects of nociceptin were antagonized by pretreatment with the ORL1 receptor antagonist, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2*H*-benzimidazol-2-one (J-113397, 0.1 mg $kg^{-1}$, i.v.). We conclude that intravenous nociceptin inhibits cough in the cat. 

*Keywords:* Cough; Antitussive; (Cat); Nociceptin; ORL1 receptor

## 1. Introduction

Antitussive drugs, such as codeine, are among the most widely administered drugs in the world (Choudry and Fuller, 1992). Codeine suppresses the responsiveness of one or more components of the central reflex pathway for cough and is intended to be useful against cough of diverse etiologies (Irwin et al., 1990). However, opioids like codeine have a side-effect profile that includes sedation, addiction potential, and constipation (Reisine and Pasternak, 1996). The discovery of a novel and effective antitussive drug that is devoid of serious side effects would fill a large unmet need in the treatment of acute or chronic cough.

Nociceptin (or orphanin FQ) is an opioid-like peptide that is the endogenous ligand for the ORL1 receptor (Meunier et al., 1995). Although this peptide shares structural homology with endogenous opioid ligands, it has a low affinity for opioid receptors and the ORL1 receptor is not considered to be a member of the family of opioid receptor subtypes (Mogil et al., 1996; Ozaki et al., 2000). Nociceptin is found in the lung (Fisher et al., 1998) and inhibits brochospasm elicited by a variety of different manipulations of airway motor and sensory nerves (Corboz et al., 2000). McLeod et al. (2001) showed that the frequency of coughing is inhibited by central (intracerebroventricular) administration of nociceptin in the guinea pig. However, it is unknown if nociceptin will inhibit cough after systemic administration. This issue is particularly important, given that nociceptin can have differential actions depending on the route of administration (Henderson and McKnight, 1997). Furthermore, while the awake guinea pig is well accepted as an animal model of cough, it typically yields information regarding the frequency of coughing but not the magnitude of this reflex. In particular, the cat has been used as a cough model that provides information regarding both the frequency and intensity of cough (Bolser et al., 1999). We speculated that systemic administration of nociceptin would inhibit cough number (an index of the frequency of coughing) and suppress the magnitude of abdominal muscle motor activity in the cat.

## 2. Methods

Cats were anesthetized with pentobarbital sodium (35 mg $kg^{-1}$, i.p.). Supplemental anesthetic was administered

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as necessary (5 mg $kg^{-1}$, i.v.). Atropine sulfate (0.3 mg kg-$^{1}$, i.v.) was administered to block reflex airway secretions. The trachea, femoral artery, and femoral vein were cannulated in all animals.

Electromyograms (EMG) from the parasternal (inspiratory) and rectus abdominis (expiratory) muscles were recorded with bipolar tungsten wire electrodes. The EMGs were amplified, filtered (0.5–10 kHz), and integrated with a resistance–capacitance circuit (100 ms time constant). The integrated EMGs were displayed on a chart recorder.

Cough was defined as a large burst of EMG activity in the diaphragm immediately followed by a burst of EMG activity in the rectus abdominis muscle (Bolser et al., 1993, 1994, 1995, 1999). Coughing was produced by mechanical stimulation of the intrathoracic trachea with a thin flexible polyethylene cannula for 10 s per stimulus trial.

The antitussive activity of the ORL1 receptor agonist nociceptin/orphanin FQ was evaluated from cumulative dose–responses obtained after i.v. administration. Control values were obtained by averaging the number of coughs during five consecutive mechanical stimulus trials after vehicle administration. One minute elapsed between stimulus trials. Stimulus trials were applied at 1-min intervals after each dose of compound for a total of five stimulus trials between doses. Approximately 5 min elapsed between each dose of compound.

In a separate group of animals, the protocol was modified to assess the effect of pretreatment with the specific ORL1 receptor antagonist 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2*H*-benzimidazol-2-one (J-113397, Corboz et al., 2000; Ozaki et al., 2000) on the antitussive effect of nociceptin/orphanin FQ. After vehicle administration, J-113397 was administered i.v. 10 min before nociceptin/orphanin FQ and the protocol was conducted as described above.

Cough measurements included cough number (the number of coughs per stimulus trial), amplitude of the integrated abdominal expiratory muscle EMG, and amplitude of the integrated inspiratory muscle EMG. The cough response after vehicle or each dose of compound was assessed by averaging cough number, abdominal muscle EMG amplitude, and inspiratory muscle EMG amplitude during the five stimulus trials.

### 2.1. Statistics

Effective doses for 50% inhibition ($ED_{50}$) of cough frequency were obtained by regression analysis of dose–response relationships. Data are expressed as mean ± S.E.M. Student's *t*-test (Mann–Whitney test for nonparametric data) or one-way analysis of variance was used to evaluate differences between means. Post hoc analysis of the data for analysis of variance was conducted by the Student's Neuman–Keuls method. $p < 0.05$ was considered significant.

### 2.2. Compounds

Nociceptin/orphanin FQ was provided by Schering-Plough Research Institute. Atropine sulfate was obtained from Sigma (St. Louis, MO, USA). Atropine sulfate and nociceptin/orphanin FQ were dissolved in physiological saline. Doses were calculated as their free base.

## 3. Results

During the control period, cough number averaged 7 ± 1 per mechanical stimulation trial. Control values of the rectus abdominis EMG during cough averaged 61 ± 7% of the maximum burst amplitude, and parasternal EMG magnitude averaged 65 ± 4% of the maximum burst amplitude.

An example of the effect of nociceptin on the cough motor pattern is shown in Fig. 1. Nociceptin (1 mg $kg^{-1}$, i.v.) decreased both the number of coughs and the magnitude of the abdominal EMG bursts during mechanical stimulation of the intrathoracic trachea, but at this dose it

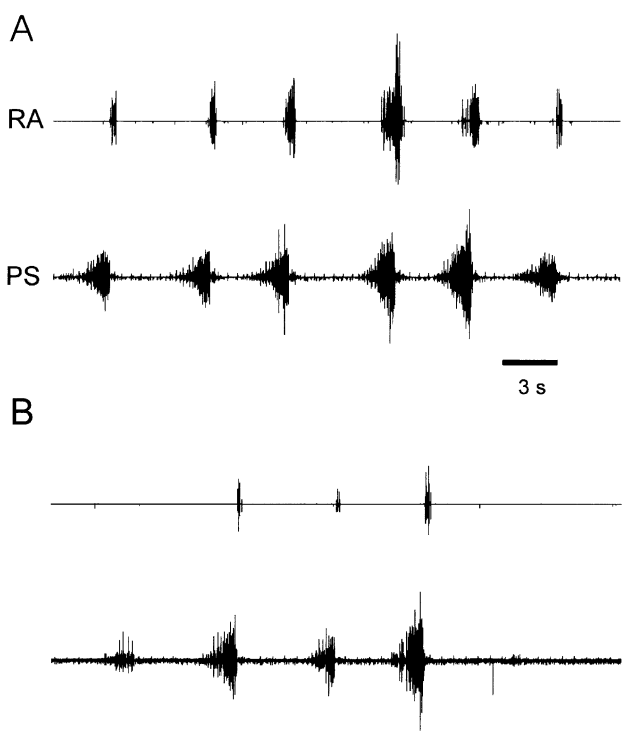


Fig. 1. Example of the antitussive effect of intravenous nociceptin on mechanically induced cough. RA—rectus abdominis EMG, PS—parasternal EMG. In panel A, the cough response (six coughs) to a single tracheal mechanical stimulation trial is shown. In panel B, the cough response (three coughs) after a cumulative dose of 1.0 mg $kg^{-1}$ (i.v.) of nociceptin is shown. Note the decreased magnitude of the rectus abdominis EMG during cough, but relatively little change in parasternal EMG elicited by nociceptin.

had relatively little effect on the magnitude of the parasternal muscle EMG (Fig. 1).

Fig. 2 shows the cumulative dose–response relationships for the antitussive effect of nociceptin/orphanin FQ (0.001–3.0 mg $kg^{-1}$, i.v. $n = 5$) on cough number, rectus abdominis EMG amplitude, and parasternal EMG amplitude during cough. Nociceptin/orphanin FQ inhibited cough number in a dose-dependent manner ($ED_{50} = 0.056$ mg $kg^{-1}$) with a maximum inhibition of approximately 80% (Fig. 2). Significant differences for the effect of nociceptin on cough number also existed between the 0.001 mg $kg^{-1}$ dose and the 0.01 ($p < 0.01$), 0.03 ($p < 0.01$), 0.1 ($p < 0.01$), 0.3 ($p < 0.001$), 1.0 ($p < 0.001$), and 3.0 ($p < 0.001$) mg $kg^{-1}$ doses. The inhibition of cough number by nociceptin also was significantly different at the 0.003 mg $kg^{-1}$ dose relative to the 0.3 ($p < 0.05$), 1.0 ($p < 0.05$), and 3.0 ($p < 0.001$) mg $kg^{-1}$ doses. Nociceptin also significantly inhibited the rectus abdominis EMG amplitudes during cough at the 0.3, 1.0, and 3.0 mg $kg^{-1}$ doses ($p < 0.05$ relative to vehicle) with a maximum inhibition of approximately 50%. Significant differences for the effect of nociceptin on rectus abdominis amplitude also existed between the 0.001 mg $kg^{-1}$ dose and 0.3, 1.0, and 3.0 mg $kg^{-1}$ doses ($p < 0.05$). However, there was no significant effect of nociceptin on parasternal EMG amplitude during cough (Fig. 2, $p < 0.34$). One animal was excluded from analysis because it began vomiting after the 0.01 mg $kg^{-1}$ dose.

The ORL1 receptor antagonist, J-113397, was used to evaluate the specificity of the nociceptin/orphanin FQ antitussive effect. At a dose of 0.3 mg $kg^{-1}$ (i.v.), J-113397 alone inhibited cough in two animals by 24% and 29%, respectively. At a dose of 0.1 mg $kg^{-1}$ (i.v.), J-113397 inhibited cough number in three animals by 37%, 33%, and 100% respectively. These five animals were excluded from further analysis. J-113397 (0.1 mg $kg^{-1}$, i.v.) had no effect on cough number in four animals ($41 \pm 8$ coughs per five trials for vehicle, $43 \pm 11$ coughs per five trials for J-113397, $p < 0.43$). In these four animals in which J-113397 did not inhibit cough, pretreatment with this compound (0.1 mg $kg^{-1}$, i.v.) significantly antagonized the antitussive effect of nociceptin/orphanin FQ (0.01–1.0 mg $kg^{-1}$, i.v., Fig. 3).

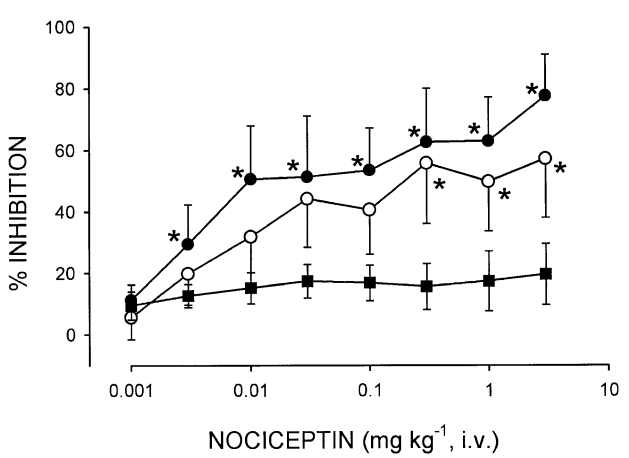


Fig. 2. Dose–response for the influence of nociceptin on cough number (●), rectus abdominis EMG amplitude (○), and parasternal EMG amplitude (■) during cough. * $p < 0.05$ relative to vehicle.

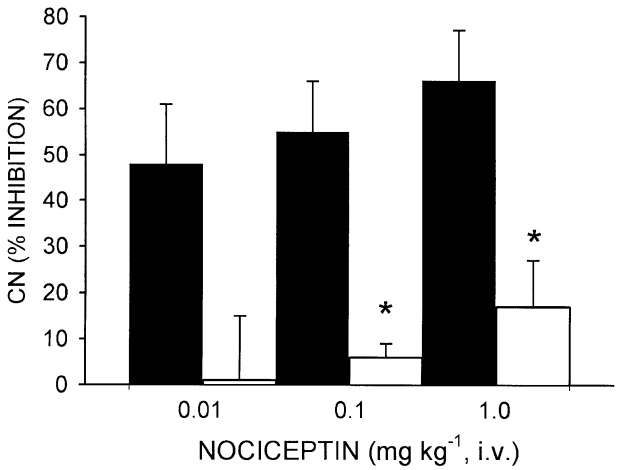


Fig. 3. The antitussive effect of nociceptin is antagonized by J-113397 (0.01 mg $kg^{-1}$, i.v.). The solid bars represent nociceptin alone ($n = 5$) and the open bars represent nociceptin plus J-113397 ($n = 4$). CN = cough number. * $p < 0.05$ relative to nociceptin.

## 4. Discussion

The major findings of this study are that intravenous nociceptin/orphanin FQ inhibited several parameters of cough in a dose-dependent manner in the cat. The dose–response relationship for nociceptin/orphanin FQ was shifted to the right by pretreatment with the ORL1 receptor antagonist, J-113397.

In our study, nociceptin/orphanin FQ, an ORL1 receptor agonist, inhibited mechanically induced cough in the cat and this effect was antagonized by the ORL1 receptor antagonist, J-113397. These findings are consistent with the antitussive effect of nociceptin/orphanin FQ found in the guinea pig (McLeod et al., 2001) and indicate that ORL1 receptors may have an important regulatory role in the production of cough. The site of action of nociceptin/orphanin FQ to inhibit cough is unknown. However, this compound is a peptide (Meunier et al., 1995) and most likely does not penetrate the central nervous system readily after intravenous administration. This characteristic of the compound is consistent with a peripheral action, but nociceptin can inhibit cough when administered either intravenously or centrally in the guinea pig (McLeod et al., 2001). Additional studies must still be performed to definitively determine the site of action of intravenous nociceptin/orphanin FQ in the cat.

Intense expiratory motor activation during repetitive coughing can lead to serious health consequences, such as

broken ribs, ruptured abdominal muscles, and pneumothorax (Braman and Corrao, 1987). It is therefore important to understand the effects of putative antitussive drugs on both the frequency and intensity of coughing. In the guinea pig, the activity of putative antitussive drugs is most often evaluated by monitoring changes in cough number in response to inhalation of irritant aerosols, such as capsaicin (Bolser et al., 1993, 1994, 1995, 1997; Karlsson et al., 1990). Indeed, McLeod et al. (2001) showed that central administration of nociceptin reduced the number of coughs in response to capsaicin inhalation in the guinea pig. Our findings both confirm and extend the work of McLeod et al. (2001) by showing that nociceptin inhibits cough number as well as the magnitude of abdominal EMGs during cough in the cat. Furthermore, our findings indicate that nociceptin in addition to inhibiting cough induced by chemical stimuli (McLeod et al., 2001) can suppress mechanically induced cough. The extent to which mechanically and chemically (capsaicin) induced cough can be mediated by different peripheral and/or central mechanisms is not completely understood. However, irritant aerosols are delivered by inhalation, which would allow them to affect sensory afferents from both the larynx and tracheobronchial airways, whereas our mechanical stimulus was limited to the intrathoracic trachea. There are significant differences between the mechanical characteristics of laryngeal and tracheobronchial cough and their relative sensitivities to suppression by codeine (Korpas and Tomori, 1979). Our results are specific to tracheobronchial cough. The influence of nociceptin on laryngeal cough is unknown.

Nociceptin did not suppress the increase in inspiratory muscle EMG that occurs during cough. This observation supports our previous findings (Bolser et al., 1999) that several different classes of antitussive drugs suppress cough number and the magnitude of the abdominal muscle EMG without altering the magnitude of the inspiratory component of cough. The antitussive drugs utilized in our previous study were administered centrally (Bolser et al., 1999). The extent to which the effect of nociceptin in the present study was limited to a peripheral site of action is unknown. However, our findings raise the question whether suppression of tracheobronchial cough in this model is associated with a similar phenotype of response regardless of the site of action of the drug.

J-113397 paradoxically inhibited cough in some animals when administered intravenously. This action of J-113397 limited its usefulness as an ORL1 receptor antagonist in this model. The antitussive action of this compound is unlikely to be related to its activity as an ORL1 receptor antagonist. This compound appears to have binding activity at $\sigma$ receptors ($< 10$ nM, R. McLeod, personal communication) and sigma receptor agonists are well known as antitussive agents (Kamei et al., 1992). While we favor the aforementioned explanation, there is as yet no published information supporting $\sigma$ receptor binding activity of this compound. There is at least one other alternative explanation that could account for our observations. J-113397 may be a mixed agonist/antagonist in the cat. To our knowledge, no one has determined the binding specificity of this compound in cat tissue.

In summary, nociceptin inhibited mechanically induced cough in a dose-dependent manner in the cat and this effect was blocked by J-113397, a nociceptin antagonist. Nociceptin specifically inhibited cough number and expiratory abdominal muscle EMG amplitude, but did not alter inspiratory muscle EMG amplitude.

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