ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10): 945-948
http://www.chinaphar.com


# Nociceptin inhibits electric field stimulation-induced cholinergic constrictions in rat airways

WU Yin[1], FANG Li-Ben, YANG Qiu-Huo
(*Department of Pharmacology, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

**KEY WORDS** nociceptin; kappa opioid receptors; trachea; bronchi; electric stimulation; cholinergic fibers

## ABSTRACT

**AIM**: To study the effect of nociceptin (orphanin FQ), a newly discovered heptadecapeptide, on cholinergic constrictions in isolated trachea and bronchus of rat. **METHODS**: The electric field stimulation (EFS) induced a monophasic constriction, which was due to an activation of the cholinergic nerves. **RESULTS**: Nociceptin 0.001 - 0.1 μmol/L inhibited cholinergic constriction in a concentration-dependent manner. $IC_{50}$ (95 % of confidence limits) were 0.06 (0.04 - 0.08) μmol/L and 0.07 (0.05 - 0.1) μmol/L in tracheae and bronchi respectively. The constrictions inhibited by nociceptin 0.01 μmol/L in tracheae and bronchi were (58 ± 32) % and (60 ± 26) % respectively compared with the control, in which nociceptin was not applied. After pretreatment with naloxone 0.1 μmol/L, the constrictions were (60 ± 19) % and (54 ± 20) % ($P > 0.05$ *vs* the above figures). However, the constrictions induced by exogenous acetylcholine were unaffected by nociceptin 0.01 μmol/L. κ-Opioid receptor agonist, U-50488H (0.01-1 μmol/L) did not affect the EFS-induced cholinergic constrictions in rat airways. **CONCLUSION**: Nociceptin inhibits EFS-induced cholinergic constriction, which is not affected by naloxone in rat airways.

## INTRODUCTION

Nociceptin[1] or Orphanin FQ[2] with an amino acid sequence Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln, is a heptadecapeptide isolated from the brain. The structure of nociceptin shows similarities with that of mammalian opioid peptide dynorphin A, except Phe1 which is essential for activating μ-, δ-, or κ- opioid receptor. Nociceptin has been suggested to be a putative ligand for the "orphan" opioid receptor termed the opioid-receptor-like 1 ($ORL_1$) receptor. The $ORL_1$ receptor resembles opioid receptors in structure, particularly the κ-opioid receptor[3], and is negatively coupled with adenylate cyclase through a G protein.

However, despite the structural similarities of nociceptin with dynorphin[4], the function and functional sites of opioids are pharmacologically different from that of nociceptin. *In vivo*, nociceptin induces hyperalgesia in the brain but analgesia in the spinal cord of rats[5]. *In vitro*, nociceptin inhibits neurogenic inflammation by reducing peripheral sensory neurotransmissions[6].

In an indirect investigation, the result showed that opioid inhibits presynaptic cholinergic neural responses in guinea pig airways mainly via an action on μ-opioid receptor[7]. While direct studies also demonstrated that nociceptin is able to inhibit electrical field stimulation (EFS)-induced acetylcholine (ACh) release from guinea-pig trachea[8]. However, whether the κ-opioid receptor agonist influences the cholinergic neural responses in rat airways is not certain[7,9].

In our research, we used the EFS method to investigate if nociceptin and trans-*dl*-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrroli-dinyl] cyclohexyl)-benzeneacetamide (U-50488H, κ-opioid receptor agonist) affect cholinergic activation-mediated constrictions in rat airways.

## MATERIALS AND METHODS

Nociceptin (Orphanin FQ), U-50488H, indometacin, propranolol, and acetylcholine were purchased from Sigma Co. Male Sprague-Dawley rats (220 g ± 25 g, Grade Ⅱ, Certificate № 22-9601018) were supplied by the Experimental Animal Center of School of Medicine, Zhejiang University. The isolated tracheae ring and the main bronchi ring (3 - 4 mm width) were suspended between 2 platinum electrodes in a 5-mL organ

[1] Correspondence to Dr WU Yin. Phn 86-571-721-7150. Fax 86-571-721-7125. E-mail sbms@mail.hz.zj.cn
Received 1999-09-27 Accepted 2000-04-24

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

bath containing Krebs-Henseleit (KH) solution at 37 ℃ and gassed with oxygen. The preparations were connected to force-displacement transducers for the measurement of isometric tension[10]. The tissues were equlibrated for 60 min under an initial tension of 0.5 g, and washed with fresh solution every 20 min. Propranolol 1 mmol/L and indometacin 2 mmol/L were added in the physiological solution.

As in previous reports[11,12] and our studies, EFS (24/60 v, 6 Hz, 1 ms pulse width, trains of 15-s duration) was applied at an interval of 20 min. The EFS-induced constriction was via activation of nerve terminals but not via the action on the smooth muscles. Taking the first two EFS as control, nociceptin was added five min before EFS. While in another experiment, naloxone was added five min before the addition of nociceptin. $BaCl_2$ 30 mmol/L was added at the end of every experiment to the maximum constriction response. The stimulator (Model JJC-2) was made in Shanghai. The recorders were manufactured by Shanghai Dahua Factory (Model XWT-264).

The constrictive tension of the airway smooth muscles was expressed as mg ($\bar{x} \pm s$) or as percentage of $BaCl_2$-induced constriction. Differences were analyzed by paired $t$ test or one-way ANOVA. $IC_{50}$ (95 % of confidence limits) were calculated and compared by weighted probit analysis of Bliss method.

## RESULTS

EFS elicited a monophasic constrictive response of tracheal and bronchial rings. The pretreatment with atropine 1 μmol/L abolished the constriction of preparations. Nociceptin (0.01, 0.03 μmol/L) did not affect the resting tension of tracheal and bronchial rings. Nociceptin 0.01 μmol/L inhibited the constriction of tracheae ($P < 0.01$) or bronchi ($P < 0.01$) (Tab 1). $IC_{50}$ (95 % of confidence limits) were 0.06 (0.04 – 0.08) μmol/L and 0.07 (0.05 – 0.1) μmol/L respectively. But U-50488H (0.001 – 1 μmol/L) did not significantly influence the constriction of tracheae and bronchi (Tab 2).

After treating the bronchi with naloxone 0.1 μmol/L, an opioid receptor antagonist, nociceptin 0.01 μmol/L still significantly inhibited the constriction of the smooth muscles in airways (Tab 3).

Exogenous ACh 0.01 – 1 μmol/L obviously induced constriction of the trachea and bronchus preparation. Pretreatment with nociceptin 0.01 μmol/L did not affect the constriction induced by exogenous ACh (Tab 4).

## DISCUSSION

Our results confirmed that U-50488H, κ-opioid receptor agonist, did not affect the cholinergic constriction of rat airways in both 24 v and 60 v EFS. It indicated that κ-opioid receptor was not involved in the EFS-induced constriction. This conclusion was in agreement with the results acquired in guinea-pig airways[7].

We have demonstrated that lower concentration of nociceptin (0.01 – 0.1 μmol/L) obviously inhibited EFS-induced cholinergic activated-constriction of smooth muscles in rat airways. Moreover, the inhibition elicited by 0.01 μmol/L nociceptin was not affected by 0.1

**Tab 1. EFS-induced constriction after pretreatment with nociceptin 0.001 – 0.1 μmol/L. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.**

| | Nociceptin/μmol·L$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.001 | 0.003 | 0.01 | 0.03 | 0.1 |
| Tracheae | | | | | | |
| Constriction/mg | 282 ± 104 | 274 ± 106$^a$ | 245 ± 113$^a$ | 188 ± 132$^a$ | 151 ± 125$^b$ | – |
| $n$ | 8 | 8 | 8 | 8 | 8 | – |
| % of control | – | 97 ± 9$^a$ | 84 ± 15$^a$ | 58 ± 32$^c$ | 44 ± 31$^c$ | – |
| % of $BaCl_2$ | 72 ± 17 | 69 ± 15$^a$ | 60 ± 17$^a$ | 42 ± 24$^c$ | 32 ± 23$^c$ | – |
| Bronchi | | | | | | |
| Constriction/mg | 342 ± 92 | 315 ± 90$^a$ | 280 ± 115$^a$ | 217 ± 124$^b$ | 176 ± 126$^c$ | 209 ± 100$^b$ |
| $n$ | 10 | 10 | 10 | 10 | 10 | 7 |
| % of control | – | 92 ± 11$^a$ | 81 ± 24$^b$ | 60 ± 26$^c$ | 48 ± 29$^c$ | 55 ± 19$^c$ |
| % of $BaCl_2$ | 71 ± 18 | 66 ± 21$^a$ | 58 ± 22$^a$ | 44 ± 21$^c$ | 31 ± 23$^c$ | 38 ± 16$^c$ |

**Tab 2. EFS-induced constriction (mg) after pretreatment with U-50488H (0.01 - 1 μmol/L). $^aP > 0.05$ compared with the control.**

| | | U-50488H/μmol/L | | | |
|---|---|---|---|---|---|
| | *n* | Control | 0.01 | 0.1 | 1 |
| Tracheae | | | | | |
| Constriction/mg | | | | | |
| 24 v* | 9 | 195 ± 109 | – | 216 ± 114[a] | 175 ± 142[a] |
| 60 v** | 8 | 310 ± 112 | 292 ± 115[a] | 286 ± 122[a] | 295 ± 142[a] |
| Bronchi | | | | | |
| Constriction/mg | | | | | |
| 24 v* | 5 | 222 ± 104 | – | 220 ± 124[a] | 183 ± 123[a] |
| 60 v** | 10 | 285 ± 89 | 268 ± 85[a] | 247 ± 91[a] | 233 ± 115[a] |

*: EFS is 24 v, 1 ms, 6 Hz. **: EFS is 60 v, 1 ms, 6 Hz.

**Tab 3. EFS-induced constriction of bronchi (+ nociceptin 0.01 μmol/L), after pretreatment with naloxone (0.1 μmol/L). $^bP < 0.05$, $^cP < 0.01$ *vs* control; $^dP > 0.05$ *vs* nociceptin.**

| | Control | Nociceptin 0.01 μmol/L | Naloxone 0.1 μmol/L + nociceptin 0.01 μmol/L |
|---|---|---|---|
| Constriction/mg | 342 ± 92 | 217 ± 124[b] | 210 ± 113[bd] |
| *n* | 10 | 10 | 7 |
| % of $BaCl_2$ | 71 ± 18 | 44 ± 21[c] | 42 ± 16[bd] |

**Tab 4. ACh-induced constriction of tracheae and bronchi with or without addition of nociceptin 0.01 μmol/L. $^aP > 0.05$ *vs* control.**

| | | Acetylcholine/μmol·L$^{-1}$ | | |
|---|---|---|---|---|
| | *n* | 0.01 | 0.1 | 1 |
| Constriction/mg | | | | |
| Control | 4 | 6.3 ± 3.2 | 201 ± 228 | 309 ± 168 |
| Nociceptin | 5 | 3.0 ± 2.1[a] | 165 ± 156[a] | 273 ± 108[a] |
| % of $BaCl_2$ | | | | |
| Control | 4 | 1.26 ± 0.58 | 33 ± 29 | 56 ± 15 |
| Nociceptin | 5 | 0.66 ± 0.41[a] | 33 ± 28[a] | 57 ± 13[a] |

μmol/L naloxone (nonselective opioid receptor antagonist). These results indicated that nociceptin biologic activity was not mediated by classical opioid receptors. Nociceptin is an endogenous ligand for the $ORL_1$ receptor. The $ORL_1$ receptor exists widely in central and peripheral nervous systems. Nociceptin is abundent in the rat lung[13]. These findings hint us that nociceptin may have important neuromodulatory role in the lung. In order to confirm the hypothesis that nociceptin is acting on $ORL_1$ receptor, a selective antagonist of nociceptin must be found.

ACh is released from parasympathetic nerves. Exogenous ACh induced a constriction of tracheal and bronchial preparation in a concentration-dependent manner. Nociceptin 0.01 μmol/L did not affect the ACh-induced constriction of smooth muscle in airways. These results further confirmed that nociceptin might inhibit constriction through presynaptic mechanism, but not through direct action on the smooth muscles.

According to the previous reports[14], similar to classical opioids, nociceptin activates an inwardly rectifying $K^+$ channel conductance and suppresses the high-voltage-activated $Ca^{2+}$ channel conductance by G protein, then modulates neural cells' excitability and neurotransmission release. Nevertheless, nociceptin also suppresses low-voltage-activated, transient calcium current in acutely dissociated rat dorsal root ganglion neurons, which is not shared by opioids and is a G protein independent effect. This may reflect different mechanisms between nociceptin and opioids. Further investigation about effect of nociceptin at a cellular level is in progress.

In conclusion, the present study indicates that the inhibitory effects of nociceptin on EFS-induced cholinergic constriction is not mediated by the classical opioids and κ-opioid receptor is not involved in the cholinergic constriction in rat airways.

**ACKNOWLEDGMENTS** We appreciate Prof Lu-Yuan LEE (University of Kentucky, USA) for his support in providing certain kind of experimental chemicals.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

## REFERENCES


1 Meunier JC, Mollereau C, Toll L, Suaudeau C, Molsand C, Alvinerle P, *et al*. Isolation and structure of the endogenous agonist of opioid receptor-like $ORL_1$ receptor. Nature 1995; 377: 532 - 5.

2 Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, *et al*. Orphanin FQ: A neuropeptide that activates an opioidlike G protein-coupled receptor. Science 1995; 270: 792 - 4.

3 Mollereau C, Parmentier M, Mailleux P, Butour J-T, Moisand C, Chalon P, *et al*. $ORL_1$, a novel member of the opioid receptor family: cloning, functional expression and localization. FEBS Lett 1994; 341: 33 - 8.

4 Goldstein A, Fischli W, Lowney LI, Hunkapiller M, Hood L. Porcin pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. Proc Natl Acad Sci USA 1981; 78: 7219 - 23.

5 Tian JH, Xu W, Zhang W, Fang Y, Han JS. Orphanin FQ antagonize the effect of electroanalgecia in the brain but enhance it in the spinal cord. Acupunct Res 1997; 22: 34 - 5.

6 Helyes Z, Nemeth J, Pinter E, Szolcsanyi J. Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. Br J Pharmacol 1997; 121: 613 - 5.

7 Belvisi MG, Stretton CD, Barnes PJ. Modulation of cholinergic neurotransmission in guinea-pig airways by opioids. Br J Pharmacol 1990; 100: 131 - 7.

8 Patel HJ, Giembycz MA, Spicuzza L, Barnes PJ, Belvisi MG. Naloxone-insensitive inhibition of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by the novel opioid, nociceptin. Br J Pharmacol 1997; 120: 735 - 6.

9 Zappi L, Nicosia F, Rocchi D, Song P, Rehder K. Opioid agonists modulate release of neurotransmitters in bovine trachealis muscle. Anesthesiology 1995; 83: 543 - 51.

10 Fang LB, Yang QH, Bian RL. Mediator difference in contractions between trachea and bronchus of guinea pig induced by stimulation of C-fibers *in vitro*. Acta Pharmacol Sin 1994; 15: 436 - 8.

11 Knott PG, Fernandes LB, Henry PJ, Gddie RG. Influence of endothelin-1 on cholinergic nerve-mediated contractions and acetylcholine release in rat isolated tracheal smooth muscle. J Pharmacol Exp Ther 1996; 279: 1142 - 7.

12 Szarek JL, Spurlock B. Antagonism of cholinergic nerve-mediate contractions by the sensory nerve inhibitory system in rat bronchi. J Appl Physiol 1996; 81: 260 - 5.

13 Li X, Fei YX, Ye JX, Pang YZ, Zhang ZK, Tang CS. Distribution of Orphanin FQ in mouse's and rat's tissues. J Beijing Med Univ 1997; 29: 385.

14 Abdulla FA, Smith PA. Nociceptin inhibits T-type $Ca^{2+}$ channel current in rat sensory neurons by a G-protein-independent mechanism. J Neurosci 1997; 17: 8721 - 8.


## 痛啡肽抑制电场刺激引起的大鼠气道胆碱能收缩反应

吴 茵，方理本，杨秋火 （浙江大学医学院基础药理学教研室，杭州 310006，中国）

**关键词** 痛啡肽；κ阿片受体；气管；支气管；电刺激；胆碱能纤维

**目的**：研究痛啡肽(nociceptin)对大鼠离体气管/支气管的胆碱能神经兴奋所致收缩的抑制作用. **方法**：记录电场刺激引起胆碱能神经兴奋所致的标本收缩张力，了解 nociceptin 的作用. **结果**：Nociceptin 0.001 - 0.1 μmol/L 可抑制标本的胆碱能收缩，其 $IC_{50}$(95 % 的可信限)分别是 0.06 (0.04 - 0.08) μmol/L 和 0.07 (0.05 - 0.1) μmol/L. 在气管和支气管上，nociceptin 0.01 μmol/L 的抑制率分别是：(58 ± 32) % 和 (60 ± 26) %；预用纳洛酮 0.1 μmol/L 后，nociceptin 的抑制率为：(60 ± 19) % 和 (54 ± 20) % ($P > 0.05$). Nociceptin 0.01 μmol/L 不影响外源性乙酰胆碱引起的气道标本收缩. κ阿片受体激动剂 U-50488H 0.01 - 1 μmol/L 不影响电场刺激引起的大鼠气道胆碱能收缩. **结论**：痛啡肽抑制电刺激引起的大鼠气道胆碱能收缩反应，且不受纳洛酮影响.

（责任编辑 刘俊城）