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Inhibition by nociceptin on excitatory non-adrenergic non-cholinergic response in guinea pig airways

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KEY WORDS nociceptin; kappa opioid receptors; bronchi; smooth muscle; guinea pigs; electric stimulation

ABSTRACT

AIM: To study the effect of nociceptin (NC), a newly discovered heptadecapeptide, and U-50488H, a kappa-opioid receptor agonist, on excitatory non-adrenergic non-cholinergic (eNANC) constriction responses in guinea pig isolated bronchus. **METHODS**: An eNANC response was induced by electric field stimulation (EFS) in the preparation via activation of the sensory nerve terminals. The effect of NC and U-50488H was analyzed on the response. **RESULTS**: Nociceptin 0.001 - 0.1 $\mu mol \cdot L^{-1}$ inhibited the eNANC constriction which was induced by EFS but not by capsaicin in guinea pig bronchus. The constriction inhibited by NC 0.01 $\mu mol \cdot L^{-1}$ was (43 ± 31) % compared with the control. After pretreatment with naloxone 0.1 $\mu mol \cdot L^{-1}$, the constriction was inhibited by (46 ± 28) %, without marked change compared with the above figure. IC_{50} (95 % of confidence limits) was 6.12 (3.8 - 9.9) $nmol \cdot L^{-1}$. U-50488H also inhibited the EFS-evoked eNANC constriction and the effect was abolished after pretreatment with naloxone. IC_{50} (95 % of confidence limits) was 1.08 (0.5 - 2.2) $\mu mol \cdot L^{-1}$. Capsaicin 0.01 - 1 $\mu mol \cdot L^{-1}$ caused a cumulative constriction response in the preparation. Moreover, the effect of capsaicin was not affected by pretreatment with NC 0.01 $\mu mol \cdot L^{-1}$ or U-50488H 0.1 $\mu mol \cdot L^{-1}$. The constriction induced by exogenous neurokinin A, were also unaffected by treatment with NC 0.01 $\mu mol \cdot L^{-1}$ or U-50488H 0.1 $\mu mol \cdot L^{-1}$ in isolated bronchus. **CONCLUSION**: Nociceptin inhibits EFS-induced eNANC constriction, which is not reversed by naloxone, while U-50488H inhibits EFS-induced eNANC response via acitivation of opioid receptor in guinea pig airways.

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INTRODUCTION

Nociceptin is a novel neuropeptide of the opioid peptide family recently identified as the endogenous ligand of the opioid receptor-like "orphan" receptor[1]. Despite structural homologies of both nociceptin (NC) and its receptor with peptides and receptors of the opioid family, there are many differences in their effects. *In vivo* studies in mice have demonstrated that NC has central hyperalgesic properties[1,2]. While at the spinal level in rats, it has antinociceptive properties similar to opioids[3].

Pharmacological studies *in vivo* indicate that NC exerts its effects predominantly in the central nervous system. However, much evidence show that in the PNS, NC inhibits electric field stimulation (EFS)-induced release of acetylcholine in guinea pig trachea[4] and rat airways[5].

In the airways, local tachykinin release from airway sensory nerves produces symptoms of inflammation and thus has been related to the pathophysiology of the asthmatic reaction[6]. EFS-induced excitatory non-adrenergic non-cholinergic (eNANC) responses are mediated by the release of tachykinins such as substance P and neurokinin A from C-fibers in guinea pig isolated bronchus[7]. Nociceptin also inhibits mediator release from sensory nerves in the guinea pig renal pelvis[8]. Opioids inhibit EFS-induced tachykinergic contractions in the airways by a naloxone-sensitive mechanism mainly mediated by μ-opioid receptor[9].

In the present study, we have investigated the effects of NC and *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrroli-dinyl)cyclohexyl]-benzeneacetamide (U-50488H, κ-opioid receptor agonist) on EFS- and capsaicin-induced eNANC constrictions in guinea pig isolated bronchial preparations.

MATERIALS AND METHODS

Materials Guinea pigs (ZMU: DHP1, 300 g ± 24 g, Grade Ⅱ, Certificate № 22-9601018), which were

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supplied by the Experimental Animal Center of Medicine, Zhejiang University, were stunned and exsanguinated. The isolated bronchi rings (3 – 4 mm width) were suspended between two platinum ring electrodes in 5-mL organ bath containing Krebs' solution which was bubbled with O_2 95 % and CO_2 5 %, pH 7.4 and maintained at 37 ℃. The tissues were allowed to equilibrated for 60 min under a resting tension of 0.5 g, and washed with fresh solution every 15 – 20 min. The preparations were connected to force-displacement transducers for the measurement of isometric tension[10]. Propranolol (1 μmol·L^{-1}), indomethacin (2 μmol·L^{-1}) and atropine (1 μmol·L^{-1}) were present in the Krebs' solution during the experiment.

EFS (48 V, 0.8 ms pulse width, 15 Hz, trains of 15 s duration)[10] were applied at an interval of 20 min. The effect by the first two EFS was taken as a control, NC was added 5 min before EFS. While in another experiment, naloxone was added 5 min before the addition of NC. $BaCl_2$ 30 mmol·L^{-1} is added at the end of every experiment. In other experiments, capsaicin (0.01, 0.03, 0.1, 0.3, 1 μmol·L^{-1}) was added sequentially to the bath solution. When one constriction response under certain concentration of capsaicin reached its plateau, a higher concentration of capsaicin was added. Nociceptin or U-50488H was added 5 min prior to the addition of capsaicin. $BaCl_2$ 30 mmol·L^{-1} was added at the end of every experiment.

Nociceptin (Orphanin FQ), U-50488H, capsaicin, neurokinin A, indomethacin, and propranolol were purchased from Sigma Co. The constriction tension of the preparation was expressed as mg ($\bar{x} \pm s$) or as percentage of $BaCl_2$ induced constriction. Differences between means were analyzed by paired t test or one-way ANOVA. IC_{50} and EC_{50} (95 % of confidence limits) were calculated and compared by weighted probit analysis of Bliss method.

RESULTS

EFS induced an excitatory non-adrenergic non-cholinergic constriction in the guinea pig isolated bronchus. TTX (1 μmol·L^{-1}) abolished this response (Fig 1).

Nociceptin did not affect the resting tension of bronchial rings. Nociceptin 0.001 – 0.1 μmol·L^{-1} inhibited the constriction of bronchi ($P < 0.01$) in a concentration dependent manner (Fig 2, Tab 1). IC_{50} (95 % of confidence limits) were 6.12 (3.8 – 9.9) nmol·L^{-1}. The inhibitory effect of NC (0.01 μmol·L^{-1}) was not reversed by nonselective opioid receptor antagonist naloxone (0.1 μmol·L^{-1}) (Tab 2).

U-50488H 0.1 – 1 μmol·L^{-1} inhibited the constriction of bronchi (Tab 3). IC_{50} (95 % of confidence limits) was 1.08 (0.5 – 2.2) μmol·L^{-1}. But after pretreating the bronchi with naloxone 0.1 μmol·L^{-1}, the inhibitory effect of U-50488H 0.1 μmol·L^{-1} was abolished (Tab 4).

Capsaicin induced cumulative constrictor response in guinea pig isolated bronchi. EC_{50} (95 % confidence limits) was 0.1 (0.07 – 0.15) μmol·L^{-1} that was not affected in the presence of NC 0.08 (0.06 – 0.12) μmol·L^{-1}, or U-50488H 0.07 (0.05 – 0.1) μmol·L^{-1}(Tab 5).

In the presence of NC 0.01 μmol·L^{-1} or U-50488H 0.1 μmol·L^{-1}, the constriction of preparation ($n = 5$) caused by neurokinin (NKA) 0.01 μmol·L^{-1} was (32 ± 19) % or (23 ± 5) % respectively, and this value was (19 ± 1) % in the absence of NC or U-50488H. These three figures showed no significance difference ($P > 0.05$).

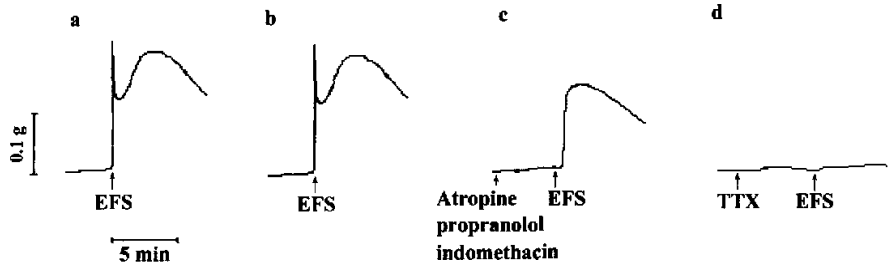

Fig 1. Excitatory non-adrenergic non-cholinergic constriction induced by EFS in the guinea pig isolated bronchus. a and b: constriction of bronchus without any antagonist; c: eNANC constriction of bronchus; d: TTX mediated abolishment of the constriction induced by EFS.

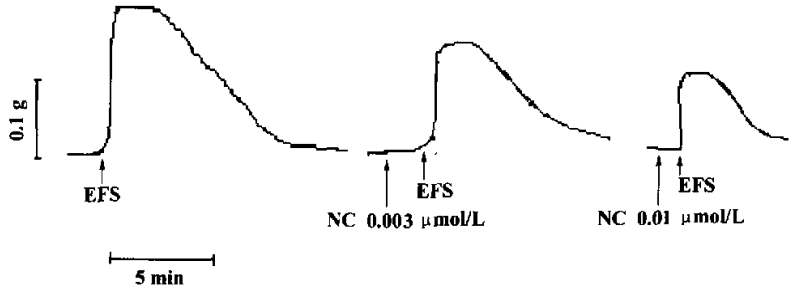

Fig 2. Inhibition by nociceptin on eNANC response in guinea pig isolated bronchus.

Tab 1. Constriction by electric field stimulation (EFS 48 V, 0.8 ms, 15 Hz) after pretreatment with nociceptin 0.001 – 0.1 μmol·L^{-1} in guinea pig isolated bronchus. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Nociceptin/μmol·L^{-1}					
	Control ($n = 16$)	0.001 ($n = 16$)	0.003 ($n = 16$)	0.01 ($n = 15$)	0.03 ($n = 8$)	0.1 ($n = 7$)
Tension/mg	198 ± 56	152 ± 67^a	127 ± 79^b	92 ± 70^c	116 ± 68^c	114 ± 67^c
% of Control	100	75 ± 24^b	60 ± 28^c	43 ± 31^c	50 ± 29^c	49 ± 25^c
% of $BaCl_2$	34 ± 11	25 ± 11^b	21 ± 12^c	15 ± 12^c	22 ± 15^c	20 ± 11^c

Tab 2. Effect of nociceptin (0.01 μmol·L^{-1}) on electric field stimulation-induced constriction of bronchi after pretreatment with naloxone (0.1 μmol·L^{-1}). $n = 16$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^dP > 0.05$ *vs* nociceptin group.

	Control	Nociceptin	Naloxone + nociceptin
Tension/mg	198 ± 56	92 ± 70^c	95 ± 63^{cd}
% of Control	100	43 ± 31^c	46 ± 28^{cd}
% $BaCl_2$	34 ± 11	15 ± 12^c	15 ± 10^{cd}

Tab 4. Electric field stimulation-induced constriction of bronchi (+ U-50488H 0.1 μmol·L^{-1}), after pretreatment with naloxone (0.1 μmol·L^{-1}). $n = 9$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$ *vs* U-50488H.

	Control	U-50488H	Naloxone + U-50488H
Tension/mg	218 ± 40	149 ± 43^c	213 ± 43^{ae}
% of Control	100	68 ± 16^c	98 ± 7^{ae}
% $BaCl_2$	38 ± 9	26 ± 10^c	38 ± 11^{ae}

Tab 3. Electric field stimulation-induced constriction after pretreatment with U-50488H (0.01 – 1 μmol·L^{-1}) in guinea pig isolated bronchus. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	U-50488H/μmol·L^{-1}			
	Control ($n = 10$)	0.01 ($n = 10$)	0.1 ($n = 10$)	1 ($n = 9$)
Tension/mg	229 ± 45	208 ± 51^a	155 ± 43^b	122 ± 55^c
% of Control	100	93 ± 11^a	70 ± 16^c	54 ± 17^c
% of $BaCl_2$	39 ± 10	36 ± 9^a	28 ± 10^b	23 ± 12^c

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Tab 5. Capsaicin evoked cumulative constriction response of brochus and effect of nociceptin or U-50488H. $n = 5$. $\bar{x} \pm s$. $^{a}P > 0.05$ *vs* control.

		Capsaicin/μmol·L^{-1} 0.01	0.1	1	EC_{50}(95 % CL)/ μmol·L^{-1}
Control	Tension/mg	37 ± 40	282 ± 87	327 ± 91	0.1 (0.07 – 0.15)
	% $BaCl_2$	12 ± 11	55 ± 4	71 ± 9	
Nociceptin 0.01 μmol·L^{-1}	Tension/mg	66 ± 38	267 ± 32	301 ± 30	0.08 (0.06 – 0.12)[a]
	% $BaCl_2$	16 ± 10	65 ± 13	73 ± 13	
U-50488H 0.1 μmol·L^{-1}	Tension/mg	51 ± 76	213 ± 97	263 ± 105	0.07 (0.05 – 0.1)[a]
	% $BaCl_2$	13 ± 16	60 ± 10	76 ± 13	

DISCUSSION

In the present study, we have demonstrated that NC, the novel opioid-like peptide, has a prominent modulatory activity on primary sensory nerve terminals in the guinea pig airway, as previously reported[11]. In bronchus, nociceptin could inhibit the excitatory NANC constriction responses induced by EFS potently and effectively, but it could not inhibit the constriction induced by capsaicin in bronchus. This inhibitory effect, was not reversed by the non-selective opioid receptor antagonist, naloxone. So it was not mediated by the classical opioid receptors. This is in consistence with the previous studies which state that NC has only very low affinity for opioid receptors. Moreover, NC is an endogenous agonist of ORL_1[1]. It seems most likely that NC acts via the ORL_1 receptor. We havealso demonstrated that NKA-induced constriction of guinea pig bronchus was not affected by nociceptin. We considered that nanomolar concentrations of NC are capable of inhibiting tachykinergic responses; along with the evidence that cell bodies in the jugular ganglion containing the ORL_1 receptor mRNA and tachykinin-containing C-fiber afferents also arise from the cell bodies[12]. Release of sensory neuropeptides following nerve depolarization, however, is mediated via opening of N-type Ca^{2+} channels both of which are regulated by opioid and NC[13,14]. So we could suspect that NC inhibited the tachykinergic transmission via activating ORL_1 receptor in the guinea pig airway.

We have investigated that U-50488H, kappa-opioid receptor agonist, also inhibited the EFS-induced eNANC constriction response in the preparation, and the inhibitory effect could be reversed by naloxone. Those findings indicated that effect of U-50488H might be mediated by classical opioid receptors. But the concentration of U-50488H which is capable of inhibiting tachykinergic response is higher than that of NC.

In other experiments, we have demonstrated NC or U-50488H had not any effect on the constriction responses elicited by capsaicin, because the release of tachykinins induced by EFS and capsaicin occurs through different mechanisms[15]. EFS stimulates the opening of tetrodotoxin-sensitive sodium channels on C-fibers, but capsaicin stimulates the non-selective ion channel. If NC and U-50488H inhibit the EFS-evoked eNANC response by a postjunctional mechanism, then it should have the same effect on the response induced by tachykinins released electrically with EFS or chemically with capsaicin. The fact that NC or U-50488H inhibited the EFS induced response selectively indicates that it probably acts prejunctionally to inhibit the action potential-driven release of tachykinins. All of them have the ability to inhibit eNANC response in the airways. Hence, if NC is devoid of severe adverse effects as opioids[16], NC should be recommended as a drug for the therapy of asthma.

In conclusion, our study demonstrated that NC inhibited the eNANC response induced by EFS in guinea pig airways, via a non-opioid-mediated mechanism, while U-50488H inhibited the EFS-evoked eNANC response via activating of opioid receptor in guinea pig airways.

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痛啡肽抑制豚鼠气道兴奋性非肾上腺素能非胆碱能反应

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关键词 痛啡肽; κ-阿片受体; 支气管; 平滑肌; 豚鼠; 电刺激

目的: 研究痛啡肽(Nociceptin, NC)及 U-50488H 对豚鼠离体支气管环的非肾上腺素能非胆碱能兴奋(eNANC)所致收缩的抑制作用. **方法**: 记录电场刺激及辣椒素引起标本 eNANC 反应的收缩张力, 了解 NC 及 U-50488H 的作用. **结果**: NC 0.001 – 0.1 μmol·L^{-1}可抑制标本的 eNANC 收缩. 与对照组相比, NC 0.01 μmol·L^{-1}抑制收缩达(43 ± 31) %; 预用纳洛酮 0.1 μmol·L^{-1}后, NC 仍抑制收缩达(46 ± 28) %. IC_{50}(95 % 可信限)是 6.12 (3.8 – 9.9) nmol·L^{-1}. U-50488H 0.01 – 1 μmol·L^{-1}可抑制 eNANC 收缩, 其 IC_{50}(95 % 可信限)为 1.08 (0.5 – 2.2) μmol·L^{-1},但是 U-50488H 0.1 μmol·L^{-1}的抑制作用可被纳洛酮 0.1 μmol·L^{-1}完全取消. 辣椒素 0.01 – 1 μmol·L^{-1}可引起 eNANC 收缩, NC 0.01 μmol·L^{-1}和 U-50488H 0.1 μmol·L^{-1}均不能明显影响辣椒素的作用. 外源性神经激肽 A 0.01 μmol·L^{-1}引起的收缩不受 NC 和 U-50488H 0.1 μmol·L^{-1}的影响. **结论**: NC 非纳洛酮敏感地抑制电场刺激引起的豚鼠气道 eNANC 反应; U-50488H 通过激动阿片受体而抑制电场刺激引起的豚鼠气道 eNANC 反应.

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