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Effect of ONO-1078, a leukotriene antagonist, on capsaicin- and substance P-induced bronchoconstriction and airway microvascular leakage in guinea pigs[1]

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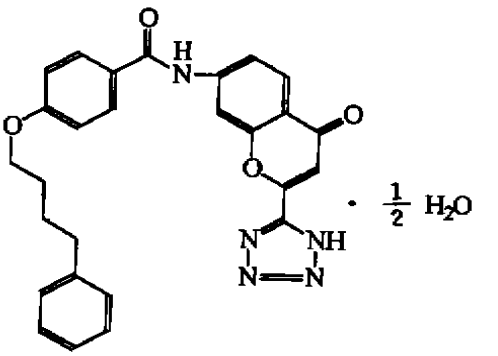
KEY WORDS ONO-1078; benzopyrans; capsaicin; substance P; bronchoconstriction; capillary permeability; bronchi; smooth muscle

AIM: To study the effect of 4-oxo-8-[*p*-(4-phenylbutyloxy) benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate (ONO-1078), a specific leukotriene antagonist, on capsaicin (Cap)-sensitive sensory nerve functions in the airways, and clarify the modulating roles of endogenous peptido-leukotrienes. **METHODS**: Changes in intrapulmonary pressure (IPP), Evans blue extravasation in airways, and contraction of bronchial smooth muscles of guinea pigs induced by Cap, substance P (SP) and leukotriene C_4 (LTC_4) were observed. **RESULTS**: Cap (0.05 $mg \cdot kg^{-1}$, iv), SP (1 $\mu g \cdot kg^{-1}$, iv) and LTC_4 (0.5 $\mu g \cdot kg^{-1}$, iv) enhanced IPP, and Evans blue extravasation in bronchi and intrapulmonary airways. ONO-1078 0.03 $mg \cdot kg^{-1}$, iv completely blocked the responses to LTC_4, attenuated those to Cap, but had no effect to SP. In isolated bronchial smooth muscles, ONO-1078 (1 $\mu mol \cdot L^{-1}$) inhibited the contractile response to Cap, but not to SP. **CONCLUSION**: ONO-1078 partly inhibits Cap-sensitive sensory nerve actions in airways, but has no direct effect on SP, a sensory neuropeptide.

4-oxo-8-[*p*-(4-phenylbutyloxy)benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate (ONO-1078), a specific antagonist for peptido-leukotrienes (LT)[1], inhibits LT- and antigen-induced bronchoconstriction and airway microvascular leakage[2,3], and also inhibits microvascular leakage induced by stimulation of capsaicin (Cap)-sensitive sensoty nerve C-fibers in the airways, heart, and skin[4,5]. The effects of LT on the airways are partly mediated by releasing sensory neuropeptides, such as substance P (SP)[6,7]. This hypothesis was supported by the fact that ONO-1078 attenuated electric stimulation of vagus (ESV)-induced airway microvascular leakage in atropine-pretreated guinea pigs[4]. This study was to clarify whether ONO-1078 modulated the release or the postsynaptic actions of sensory neuropeptides in airways, using Cap, a sensory neuropeptide releasing agent, and SP, one of the sensory neuropeptides as stimuli.



ONO-1078

MATERIALS AND METHODS

Drugs ONO-1078 (Ono Pharmaceutical Co Ltd, Osaka, Japan); Evans blue, Cap and LTC_4 (Sigma, USA); SP (Peptide Institute, Osaka, Japan); atropine sulfate (Minsheng Pharmaceutical Factory, Hangzhou, China).

Bronchoconstriction and Evans blue extravasation in airways Hartley guinea pigs of either sex weighing **326** ± *s* **65** g (*n* = **89**) were from Laboratory Animal Center of Zhejiang Medical University. Having been anesthetized with sodium pentobarbital (**30** $mg \cdot kg^{-1}$, ip), the animals were ventilated with a rodent ventilator (DH-**140**, Medical Instrumental Factory of Zhejiang Medical University, tidal volume: **5** mL, **80** breaths per min) via a tracheal cannula. Intrapulmonary pressure (IPP) was measured by a transverse

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piezoresistive pressure transducer (MPX 10DP, Motorola, USA) attached to a side arm of the tracheal cannula. The animals were serially pretreated iv with following agents: atropine (1 mg·kg^{-1}), 10 min; Evans blue (30 mg·kg^{-1}), 2 min; and ONO-1078 (0.03 mg·kg^{-1}) or solvent (5 % ethanol in saline 1 mL·kg^{-1}), 10 min. Then, Cap 0.05 mg·kg^{-1}, SP 1 μg·kg^{-1}, LTC_4 0.5 μg·kg^{-1}, or saline were injected iv. Ten minutes later, the animals were perfused with 50 mL saline iv within 1 min, and bronchi and right lower lobes of the lungs (intrapulmonary airways) were isolated. The extravasated Evans blue in the tissues was measured[6].

Bronchial smooth muscle contraction The bronchial spiral preparations were equilibrated in oxygenated Krebs solution at 37 ℃ with a resting tone of 0.67 g for 60 min with repeated washing. In the presence of ONO-1078 (1 μmol·L^{-1}) or solvent (0.001 % ethanol and 0.001 % Tween 80), gradually increasing concentrations of Cap or SP (0.1 nmol·L^{-1} to 1 μmol·L^{-1}) were added. The contractions were measured by a force displacement transducer (SMU-A, Department of Pharmacology, Shanghai Medical University).

Statistical analysis Significances of differences were determined by t test, ANOVA, or Mann-Whitney U test.

RESULTS

In the atropine-pretreated guinea pigs, the baseline values of IPP in each group before administration of Cap, SP, and LTC_4 were similar (Tab 1).

Tab 1. Baseline values of intrapulmonary pressure (IPP) in different groups. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control. ONO: ONO-1078. Cap: capsaicin. SP: substance P. LTC_4: leukotriene C_4.

	n	IPP/kPa
Control	7	1.24 ± 0.48
ONO	7	1.18 ± 0.41[a]
Cap	10	1.31 ± 0.58[a]
ONO + Cap	11	1.22 ± 0.59[a]
SP	8	1.18 ± 0.21[a]
ONO + SP	8	1.17 ± 0.17[a]
LTC_4	7	1.31 ± 0.17[a]
ONO + LTC_4	5	1.25 ± 0.09[a]

Cap elicited a biphasic increase in IPP, while SP induced a single delayed increase in IPP (Fig 1). ONO-1078 (0.03 mg·kg^{-1}) had no remarkable direct effect on IPP and Evans blue extravasation in the airways, and inhibited the increase of IPP and Evans blue extravasation in bronchi by Cap, but not by SP. On the other hand, ONO-1078 completely blocked the increases in IPP and Evans blue extravasation evoked by LTC_4 (Fig 1, Tab 2).

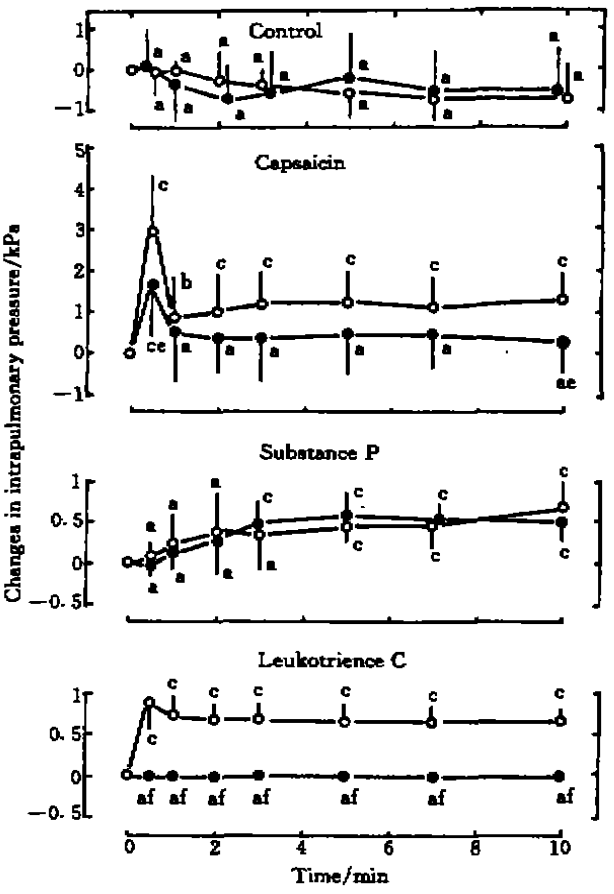

Fig 1. Effect of ONO on bronchoconstriction induced by Cap (0.05 mg·kg^{-1}, iv), SP (1μg·kg^{-1}, iv), and LTC_4 (0.5 μg·kg^{-1}) in guinea pigs. ○: solvent, ●: ONO 0.03 mg·kg^{-1}. $n = 5 - 11$ (Tab 1), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* before stimulation; $^eP < 0.05$, $^fP < 0.01$ *vs* solvent.

In the isolated bronchial smooth muscles, ONO-1078 (1 μmol·L^{-1}) also inhibited the contractile response to Cap, but not to SP (Fig 2).

DISCUSSION

ONO-1078 inhibited Cap-induced bronchoconstriction and airway microvascular leakage, but did not alter SP-induced responses, indicating that this agent modulated the release of neuropeptides from

Tab 2. Effects of ONO on Evans blue extravasation in bronchi and intrapulmonary airways increased by Cap, SP and LTC_4 in guinea pigs. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* solvent.

	n	Evan blue extravasation ($\mu g/g$ wet tissue)	
		Bronchi	Intrapulmonary airways
Control	7	10.4 ± 3.1	14.0 ± 4.2
ONO 0.03 $mg \cdot kg^{-1}$	7	13.2 ± 6.7^a	10.2 ± 3.5^a
Cap 0.05 $mg \cdot kg^{-1}$	10	78.9 ± 13.0^c	44.8 ± 8.5^c
Cap + ONO	11	55.1 ± 17.8^{cf}	36.9 ± 9.5^{cd}
SP 1.0 $\mu g \cdot kg^{-1}$	8	119.1 ± 14.2^c	33.1 ± 6.2^c
SP + ONO	8	123.9 ± 25.3^{cd}	31.3 ± 7.2^{cd}
LTC_4 0.5 $\mu g \cdot kg^{-1}$	7	101.3 ± 18.9^c	32.9 ± 11.3^b
LTC_4 + ONO	5	23.3 ± 11.3^{df}	20.9 ± 4.8^{ae}

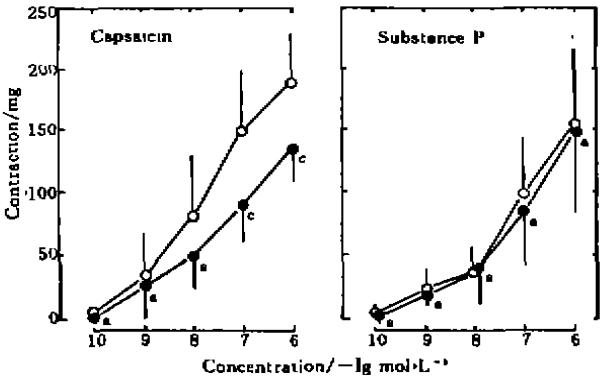

Fig 2. Effect of ONO on Cap- and SP-induced bronchial smooth muscle contraction. ○: control, ●: ONO 1 μmol $\cdot L^{-1}$; $n = 6-18$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

sensory C-fibers, not the postsynaptic effects of neuropeptides, such as SP. The inhibition of Cap-induced airway actions by ONO-1078 is likely duo to its selective effect on LT, because it completely inhibited the responses to LTC_4 at a relatively small dose ($0.03\ mg \cdot kg^{-1}$), and failed to inhibit the actions of histamine, $PGF_{2\alpha}$, arachidonic acid (Ono Pharmaceutical Co, personal communication). Thus, our data confirm that endogenous LT plays a role in sensory nerve stimulation. In the isolated guinea pig bronchial smooth muscles, another LT antagonist, SKF 104353, inhibited Cap-sensitive contractile responses to electric field stimulation[6], and LTD_4 enhanced the release of tachykinins (SP and neurokinin A) from isolated and perfused guinea pig lungs[7]. Since airway sensory C-fibers are involved in the pathogenesis of bronchial asthma[9], and LT antagonists, including ONO-1078, are effective in treating clinical asthma as a new class[10], these results explain one mechanism of the antiasthmatic actions of LT antagonists.

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白三烯拮抗剂 ONO-1078 对辣椒素和 P 物质诱导豚鼠支气管收缩和微血管渗漏的作用[1]

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关键词 ONO-1078; 苯并吡喃类; 辣椒素; P 物质; 支气管收缩; 微血管渗漏性; 支气管; 平滑肌

目的: 探讨白三烯特异性拮抗剂 4-氧-8-[对-(4-苯丁氧基)苯甲酰氨基]-2-(5-四唑基)-4H-1-苯并吡喃半水合物(ONO-1078)对气道辣椒素敏感的感觉神经功能的调节作用. 方法: 观察豚鼠肺内压(IPP)、伊文思蓝渗出量和离体支气管平滑肌收缩反应. 结果: 辣椒素(Cap, 0.05 mg·kg^{-1}, iv)、P 物质(SP, 1 μg·kg^{-1}, iv)和白三烯 C_4 (LTC_4, 0.5 μg·kg^{-1}, iv)增高 IPP 和支气管及肺内气道伊文思蓝渗出量, ONO-1078 (0.03 mg·kg^{-1}, iv)完全阻断 LTC_4 的作用, 减弱 Cap 的作用, 但不影响 SP 的作用. ONO-1078 (1 μmol·L^{-1})还显著抑制 Cap 收缩支气管平滑肌, 对 SP 无效. 结论: ONO-1078 通过抑制感觉神经肽释放而部分抑制 Cap 的作用.

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Effect of nimodipine on infectious brain edema in rabbits

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KEY WORDS *Bordetella pertussis*; brain edema; nimodipine; water; calcium; calmodulin; sodium; Evans blue

AIM: To study the effect of nimodipine (Nim) on infectious brain edema (BE). **METHODS**: An infectious BE model was induced by injection of *Bordetella pertussis* suspension (BPS) into right internal carotid artery in rabbits. Eighteen rabbits were randomly divided into 3 groups ($n = 6$). Group BE: BPS (0.6 mL·kg^{-1}) was given; group NS: normal saline was given as control; group Nim: 10 min after injection of BPS, Nim, 10 μg·kg^{-1}, was injected iv as a bolus followed by continuous infusion of 0.75 μg·kg^{-1}·min^{-1}. All the rabbits were kept under observation for 4 h. Evans blue staining was assessed; water, calcium, calmodulin (Cal), and sodium contents were determined in the right brain. **RESULTS**: Nim *vs* BE: water 82.2±1.0 % *vs* 84.4±1.2 ($P < 0.01$); calcium 10.5 ± 1.3 mmol·kg^{-1} dry tissue *vs* 17.5±1.4 ($P < 0.01$); Cal 15.9±1.8 μmol·kg^{-1} wet tissue *vs* 24.0±3.0 ($P < 0.01$); sodium 173±7 mmol·kg^{-1} dry tissue *vs* 275±38 ($P < 0.05$). No significant difference for Evans blue staining between the two groups. **CONCLUSION**: Nim had beneficial effect on the infectious BE.

Disruption of calcium homeostasis is one of the detrimental factors leading to cell death after cerebral ischemia and trauma[1]. Calcium accumulation in brain tissue plays an important role in development of infectious brain edema (BE) of rabbits[2]. Beneficial effect of calcium channel blockers after cerebral ischemia in animal and human studies have been reported[3,4], but no reports about the effect of calcium channel blockers on infectious BE have been found yet. The purpose of this study was to investigate the effect of nimodipine (Nim) on calcium accumulation and calmodulin (Cal) content in brain tissue and brain edema, using an infectious BE model in rabbits.

MATERIALS AND METHODS

The infectious BE models were induced by injection of *Bordetella pertussis* suspension (BPS) (produced by Beijing Research Institute of Biological Product, batch number 88 -

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