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Acetamide-45 inhibits histamine- and methacholine-induced contraction of isolated guinea pig trachea[1]

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LU Yun-Bi, CHEN Zhong[2], WU Ming[3] (*Department of Pharmacology, School of Medicine, Zhejiang University, Hangzhou 310031*; [3]*Department of Cardiovascular Thoracic Surgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China*)

KEY WORDS anti-allergic agents; acetamide-45; smooth muscle; trachea; muscle contraction

ABSTRACT

AIM: To investigate the effects of the new antiallergic agent *N*-(pyridin-4-yl)-(indol-3-yl) acetamide-45 (acetamide-45) on histamine- and methacholine-induced contraction of the isolated guinea pig trachea. **METHODS**: Cumulative histamine or methacholine concentration-contraction studies were carried out in the absence or presence of acetamide-45. Changes in isometric force were measured by force transducers and recorded on a multi-channel polygraph recorder. **RESULTS**: Acetamide-45 (1 – 30 μmol/L) concentration-dependently inhibited histamine- or methacholine-induced contractile response of isolated guinea pig trachea. At concentrations of 3, 10, 30 μmol/L, acetamide-45 significantly decreased maximum contractile response to histamine by 21 % – 51 %, and EC_{50} values (95 % confidence limits) were 31.1 (24.4 – 39.8), 34.7(26.8 – 45.0), and 134.4 (82.2 – 220.0) μmol/L, respectively. Similarly, acetamide-45 also inhibited the contraction induced by methacholine in isolated guinea pig trachea. **CONCLUSION**: Acetamide-45 inhibited histamine- or methacholine-induced contraction of isolated guinea pig trachea, and these effects might be non-specific for either histamine receptor or cholinoceptor.

INTRODUCTION

Asthma is a complex chronic inflammatory disease of airways that involves the activation of many inflammatory and structural cells. All of these cells release inflammatory mediators that result in the typical pathophysiologic changes of asthma[1,2]. These inflammation mediators (histamine, leukotriene, *etc*) produce many effects in the airways including bronchoconstriction, plasma exudation, mucus secretion, neural effects (such as to enhance acetylcholine release), and activation of inflammatory cells, and also play roles in remodelling of airways[3,4].

In recent years, the drug treatment of asthma has been improved by the implementation of management guidelines emphasizing the pivotal role of anti-inflammation therapy[5]. Corticosteroids are demonstrated to be used as the first line anti-inflammatory agents, but their long-term treatments have the potential dose-related systemic adverse effects[6]. So, a second line anti-inflammatory controller, eg, β_2-agonist, theophylline, leukotriene antagonists, may be needed as alternatives to monotherapy with corticosteroids[7]. However, these drugs have many defects instead of corticosteroids to treat patients with mild to moderate asthma[7]. Therefore, further studies are required to search new anti-asthma agent.

Recently, a series of new *N*-(pyridin-4-yl)-(indol-3-yl) alkylamides 44 – 84 has been introduced in the search of novel antiallergic drugs[8]. Some of these compounds have been indicated to inhibit IL-4 and IL-5 biosynthesis and histamine release. In addition, acetamide-45, which was one of the series, was synthesized lately. Initial study shows that it has more potent physiologic and pharmacologic activity than others[8]. Acetamide-45 has been previously reported to inhibit the allergent-induced late phase eosinophilia in actively sensitized guinea pigs and ovalbumin-induced rhinitis in actively sensitized rats by nasal perfusion[8], which suggested that acetamide-45 might turn into a new antiallergic agent. However, till now less was known about its effects on contraction of airways in asthma.

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[2] Correspondence to Dr CHEN Zhong. Phn 86-571-8721-7156. Fax 86-571-8721-7070. E-mail chenzfl@hotmail.com
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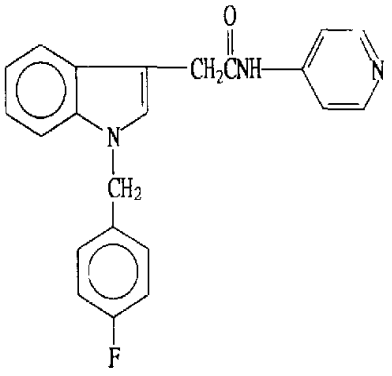
Therefore, the present study was designed to examine the possible inhibitory effect of acetamide-45 on histamine- and methacholine-induced contraction of the isolated guinea pig trachea.



MATERIALS AND METHODS

Animals Male Hartley guinea pigs (270 – 400 g, Grade Ⅱ) were purchased from Experimental Animal Center of Zhejiang University (Certificate № 22-9601018). They were housed at a constant temperature (24 ± 2) ℃ with a constant relative humidity (55 % ± 5 %), and maintained in individual cages with a 12 h light-dark cycles (lights on from 8:00 – 20:00). Water was given *ad libitum*.

Smooth muscle contraction in guinea-pig trachea Guinea pigs were killed by exsanguination under anaesthesia with pentobarbital sodium (75 mg/kg, ip). The trachea was isolated and cleaned of surrounding connective tissue, and each was cut into 8 segments. Every 2 segments (3 – 4 mm width of each) were used to make trachea chain according to the method described previously[9,10]. The trachea chain was suspended in 10-mL jacketed organ bath chamber in modified Kerbs' solution (composition in mmol/L: NaCl 118.2, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.0, glucose 10.0). Indomethacin 3 μmol/L was added to abolish the influence of cyclooxygenase products. The tissue baths were maintained at 37 ℃ and aerated with 95 % O_2/5 % CO_2. Tension was measured isometrically with force transducers and responses were recorded on a multi-channel polygraph recorder. Chains were placed under an initial tension of 1.0 g.

After an equilibration period of 60 min, two contractions were induced by histamine 3 μmol/L, then the tissue was rinsed three times with buffer. Following a period of 30 min, the chain relaxed to original baseline levels. The chains were contracted with histamine in a cumulative manner, seven concentrations between 0.3 and 300 μmol/L being used. In the other experiment, the chains were contracted with methacholine, seven concentrations between 0.1 and 100 μmol/L being used.

After the chain was rinsed three times with buffer, a further 30 min of resting period was allowed. Acetamide-45 (1, 3, 10, and 30 μmol/L) was added to the tissue bath 5 min before second histamine or methacholine stimulation. In control, acetamide-45 was replaced by its vehicle, hydrochloric acid (HCl), at the finial concentration of 9 μmol/L. All responses to histamine and methacholine, in absence or presence of acetamide-45, were expressed as percentage of maximal contraction by histamine and methacholine alone.

In another part of the experiment, the trachea chains were precontracted by histamine 300 μmol/L or methacholine 100 μmol/L after equilibration. The relaxation response to vehicle (HCl at the finial concentration of 9 μmol/L) and acetamide-45 (1, 3, 10, and 30 μmol/L) were measured at 5 min after exposure to histamine, and at 15 min after exposure to methacholine. Each experiment was performed in 4 different chains from the same animal (for three concentrations of acetamide-45 and control).

Drugs Histamine, methacholine, indomethacin, and pentobarbital sodium were purchased from Sigma Chemical Co (St Louis, USA). *N*-(pyridin-4-yl)-(indol-3-yl) acetamide-45 (acetamide-45) was kindly presented by Prof Le Baut GUILLAUME (Department of Organic Chemistry and Medical Chemistry, Faculty of Pharmacy, France). Acetamide-45 was dissolved in 0.3 mol/L HCl and diluted with double-distilled H_2O.

Data analysis All data were expressed as $x \pm s$. Difference between mean of more than two groups were analyzed with One-way ANOVA and Dunnett's test using computer software (SigmaStat 1.01 for Windows 95, 1992, Jandel Corp, USA). EC_{50} (95 % confidence limits) was calculated and compared by weighted probit analysis of Bliss method.

RESULTS

Histamine and methacholine induced concentration-dependent contraction in isolated guinea pig trachea Both histamine and methacholine

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induced concentration-dependent contraction in guinea pig trachea [EC_{50}(95 % confidence limits) and the maximal increment of trachea smooth muscle tone: histamine, 10.1 (8.3 - 12.2) μmol/L and (1.6 ± 0.3) g, n = 16; methacholine, 1.3 (1.0 - 1.6) μmol/L and (1.8 ± 0.4) g, n = 12] (Fig 1,2).

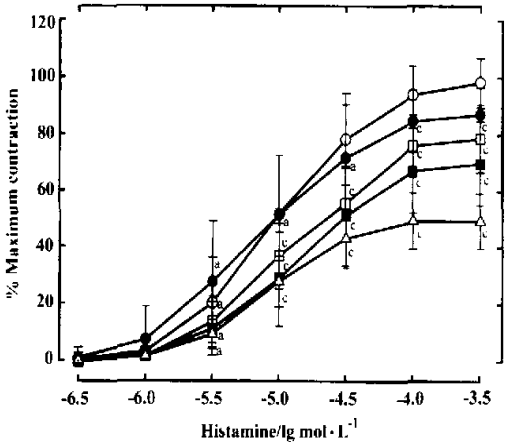

Fig 1. Effect of acetamide-45 on the contractile response of guinea pig tracheal chain to histamine by a cumulative manner in absence (○, n = 16, control) and presence of acetamide-45 (●, 1 μmol/L, n = 6; □, 3 μmol/L, n = 9; ■, 10 μmol/L, n = 9; △, 30 μmol/L, n = 9). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

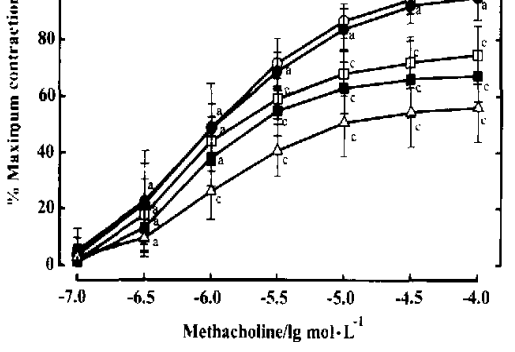

Fig 2. Effect of acetamide-45 on the contractile response of guinea pig tracheal chain to methacholine by a cumulative manner in absence (○, n = 12, control) and presence of acetamide-45 (●, 1 μmol/L, n = 6; □, 3 μmol/L, n = 6; ■, 10 μmol/L, n = 6; △, 30 μmol/L, n = 6). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Effect of acetamide-45 on histamine-induced contractile response of isolated guinea pig trachea Acetamide-45 1 - 30 μmol/L concentration-dependently inhibited histamine-induced contractile response in isolated guinea pig trachea (Fig 1). Acetamide-45 (3, 10, and 30 μmol/L) increased EC_{50} values (95 % confidence limits) of histamine to 31.1 (24.4 - 39.8), 34.7 (26.8 - 45.0), and 134.4 (82.2 - 220.0) μmol/L, respectively. Acetamide-45 (3 - 30 μmol/L) decreased maximum contractile response to the histamine by 21 % - 51 % ($P < 0.01$ *vs* control group) (Tab 1, Fig 1). In addition, acetamide-45 (1 - 30 μmol/L) had no effect on the basal tracheal tone for at least 30 min.

Tab 1. Effect of acetamide-45 on pEC_{50} values and maximal response for the histamine-induced contraction in guinea pig trachea. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Drug/ μmol·L⁻¹	n	pEC_{50}	E_{max}/%	Contraction/g
Control	16	4.97 ± 0.29	98 ± 9	1.6 ± 0.3
Acetamide-45				
1	6	4.9 ± 0.4	87 ± 9	1.3 ± 0.3
3	9	4.51 ± 0.22^c	79 ± 12^c	1.2 ± 0.3^c
10	9	4.5 ± 0.4^c	75 ± 15^c	1.1 ± 0.4^c
30	9	3.9 ± 0.4^c	49 ± 10^c	0.7 ± 0.3^c

Effect of acetamide-45 on methacholine-induced contractile response of isolated guinea pig trachea Pretreatment with acetamide-45 inhibited the contraction induced by methacholine in isolated guinea pig trachea, which was more effective than that induced by histamine (Tab 1,2). Acetamide-45 (3, 10, and 30 μmol/L) increased EC_{50} values (95 % confidence limits) of methacholine to 3.8 (2.8 - 5.3), 6.5 (4.6 - 9.3), and 19.6 (12.4 - 31.2) μmol/L ($P < 0.01$ *vs* control group), and decreased maximum contractile response to methacholine by 25 % - 44 % ($P < 0.01$ *vs* control group) (Tab 2, Fig 2).

Relaxation of acetamide-45 on histamine or methacholine precontracted trachea Cumulative concentration-response curves for histamine revealed that maximum contractions were usually obtained at 300 μmol/L, and for methacholine at 100 μmol/L. When trachea chains were treated with histamine 300 μmol/L, approximately 80 % of the maximal contraction occurred within 3 min of exposure, and reached peak in 5 - 10 min. The maximum contraction was maintained for about 15 min. Approximately 80 % of the methacholine

100 μmol/L-induced contraction occurred within 2 min of exposure, developing slowly and reaching the peak in 15 – 25 min. The maximum contraction was maintained for at least 45 min. However, acetamide-45 (1, 3, 10, 30 μmol/L) could not relax the trachea precontracted by histamine 300 μmol/L or methacholine 100 μmol/L (data not shown).

Tab 2. Effect of acetamide-45 on pEC_{50} values and maximal response for the methacholine-induced contraction in guinea pig trachea. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Drug μmol·L^{-1}	*n*	pEC_{50}	E_{max}/%	Contraction/g
Control	12	5.92 ± 0.23	99 ± 6	1.8 ± 0.4
Acetamide-45				
1	6	5.88 ± 0.27	95 ± 8	1.8 ± 0.5
3	6	5.4 ± 0.3^c	75 ± 10^c	1.3 ± 0.3^c
10	6	5.3 ± 0.4^c	67 ± 9^c	1.0 ± 0.3^c
30	6	4.6 ± 0.6^c	56 ± 12^c	0.74 ± 0.17^c

DISCUSSION

The present study showed that acetamide-45 had no effect on the basal tracheal tone and acetamide-45 inhibited both histamine- and methacholine-induced contraction of isolated guinea pig trachea. The nature of this inhibition, however, varied with concentration. Acetamide-45 1 μmol/L did not inhibit contraction stimulated by histamine or metahcholine. At higher concentrations (3 – 30 μmol/L), a nonparallel shift of the agonist concentration-response curve with concomitant lowering of maximal response was observed, suggesting either reversible noncompetitive or irreversible inhibitory effects of acetamide-45.

However, acetamide-45 (3 – 30 μmol/L) inhibited both histamine- and methacholine-induced contraction, which also suggested that the inhibitory effect of acetamide-45 is non-specific for either histamine receptor or cholinoceptor. Maybe acetamide-45 acts on the downstream of the two receptors activation. Contraction stimulated by Histamine is mainly mediated by H_1 receptor, and methacholine mainly by M_3 muscarinic cholinoceptor. H_1 receptors and M_3 receptors are coupled to phosphoinositide (PI) turnover[3,11]. The two spasmogens stimulate PI hydrolysis that leads to the increment of inositol-1, 4, 5-trisphophate (IP_3) levels followed by release of intracellular calcium ion[3,12]. The contractile response to the two spasmogens is largely independent of extracellular Ca^{2+} [3,12]. Menciu *et al*[8] assumed that acetamide-45 inhibited histamine release from most cells was involved in decrease in intracellular Ca^{2+} concentration. Therefore, the observed inhibitions of histamine- or methacholine-induced contraction by acetamide-45 in guinea pig trachea, are likely due to the possible effects on intracellular Ca^{2+} concentration.

Acetamide-45 had no effects on the isolated guinea pig trachea precontracted by histamine or methacholine. This suggested that acetamide could not relax precontracted trachea induced by cascade reaction after histamine receptor or cholinoceptor activation and acetamide-45 might have no effects on the activated downstream of the two receptors activation. Further investigation needs to elucidate the underlying mechanism.

Generally, our study provided an initial evidence that acetamide-45 inhibited histamine- and methacholine-induced contraction of isolated guinea pig trachea, and these effects might be non-specific for either histamine receptor or cholinoceptor.

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Acetamide-45 抑制组胺和乙酰甲胆碱引起的豚鼠离体气管收缩[1]

卢韵碧，陈 忠[2]，吴 明[3]（浙江大学医学院药理学教研室，杭州 310031；[2]浙江大学医学院附属第二医院胸心外科，杭州 310009，中国）

关键词 抗变态反应药；acetamide-45；平滑肌；气管；肌收缩

目的：探讨新型抗变态反应药 *N*-对氟苄基-3-[(*N*-4-吡啶)-乙酰胺]-吲哚-45（acetamide-45）对组胺和乙酰甲胆碱引起的豚鼠离体气管收缩的影响。**方法**：以 acetamide-45 预处理气管标本后，以累积剂量法给予组胺和乙酰甲胆碱，观察 acetamide-45 对组胺和乙酰甲胆碱量效曲线的影响。气管张力的变化通过换能器转变为电信号并由记录仪记录。**结果**：Acetamide-45（1 – 30 μmol/L）浓度依赖地抑制组胺和乙酰甲胆碱引起的豚鼠离体气管收缩。Acetamide-45（3，10，30 μmol/L）使组胺的量效曲线的最大效应下降了 21 % – 51 %，使组胺的 EC_{50} 值（95 % 可信限）分别增加到 31.1（24.4 – 39.8），34.7（26.8 – 45.0）和 134.4（82.2 – 220.0）μmol/L。另一方面，acetamide-45 更有效地抑制乙酰甲胆碱引起的豚鼠离体气管收缩。**结论**：Acetamide-45 抑制由组胺和乙酰甲胆碱引起的豚鼠离体气管收缩，提示 acetamide-45 的抑制作用是非特异性地作用于组胺受体或胆碱能受体。

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