Influence of epithelium removal on allergic contraction of isolated tracheal smooth muscle

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ABSTRACT Sensitized guinea pig tracheal preparations with epithelium removed were more sensitive to acetylcholine, histamine substance P, and barium chloride than those with epithelium intact. EC_{50} of them obtained in the preparations with epithelium removed dramatically decreased to 1/6 — 1/30 *vs* that obtained in epithelium intact ones. The amplitudes of contractions of epithelium-removed preparations induced by antigen-antibody reaction or electric field stimulation increased by 50 % — 100 % *vs* the control. These results indicated that the epithelium had important modulative effects on the airway, especially on its allergic contractions.

KEY WORDS acetylcholine; histamine; substance P; barium; trachea; epithelium; antigen-antibody reaction

Non-specific hyperreactivity of airway is a characteristic feature of bronchial asthma and is associated with damage to, or loss of, the epithelial cells lining the airway[1]. The epithelium influences the reactivity of many bronchomotor agonists[2-4]. Potentiation of constrictor agonists was reported in the absence of epithelium[4-6]. Non-spesific hyperreactivity might be related to the loss of airway epithelium[7]. But there was no direct evidence showing that the epithelium also had modulative effects on the allergic contraction of airway smooth muscle *in vitro*. Here, we studied whether the removal of epithelium influenced the reactivity of sensitized guinea pig tracheal smooth muscle to constrictor agonists, antigen-antibody reaction, and electric field stimulation (EFS) in order to reveal the modulative effects on allergic contraction.

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METHODS

Agents Acetylcholine (ACh) and histamine (His) were bought from Sigma; substance P (SP) and barium chloride ($BaCl_2$) were bought from Shanghai Dong Feng Biochemical Reagent Plant and Tianjin Chemical Reagent Plant, respectively.

Sensitization Sixty guinea pigs, ♀, weighing 185 ± s 18 g were sensitized by 12.5 % ovalbumin (vol/vol) in 0.9 % saline (0.1 ml, ip and sc). After 21 d, the animals were used in the following experiment.

Epithelium-removed preparations (ERP) The epithelium was removed by 2 methods. 1) Sensitized guinea pigs were stunned and exsanguinated. After the connective tissue and fat were removed the trachea was cut longitudinally opposite the band of smooth muscle. One half was kept intact as control while the epithelium in the other half was stripped off by gently rubbing the lumen with a cotton wool-tipped applicator. Either the proximal or the distal part was used randomly for epithelium removal. 2) Sensitized guinea pigs were put into a 26 cm × 26 cm × 20 cm glass container and challenged by exposure to aerosolized ovalbumin (6 % vol/vol in 0.9 % saline) for 5 min in order to induce damage or loss of epithelium lining the airways. After 48 h, the same process for tracheal preparations was accomplished. Histological examination was made to testify the epithelial removal. ERP and the control were cut in a zig-zag fashion[8]. The tissue was suspended in 5 ml Krebs solution gassed with 95 % O_2 + 5 % CO_2 at 37 ℃ and stretched with an initial tension of 1 g. After a stabilization period of over 1 h, drug effects were plotted from cumulative doses. The tension changes were

recorded by a force-displacement transducer connected to a 2-pen recorder (LM-14-224 made in Shanghai Dahua Apparatus Factory).

Antigen-antibody reaction on ERP After 60-min stabilization period, 25 % (vol/vol) ovalbumin in 0.9% saline (5 μl) was added into the bath.

Electric field stimulation The ERP was stimulated transmurally with platinum electrodes by a stimulator (DSC-FG-1) at a supermaximal voltage (100 Hz, 1 ms, 30 s).

Statistical analysis All data were expressed as $\bar{x}\pm s$. Results were analyzed by t test.

RESULTS

Histological observations showed that most epithelial cells of sensitized guinea pig tracheal preparations treated by either mechanical method or antigen challenge were severely damaged or disappeared.

ACh, His, SP, and $BaCl_2$ induced a contraction of the preparation (Fig 1). The reactivity of ERP to the contractor agonists was much higher *vs* control (intact epithelium).

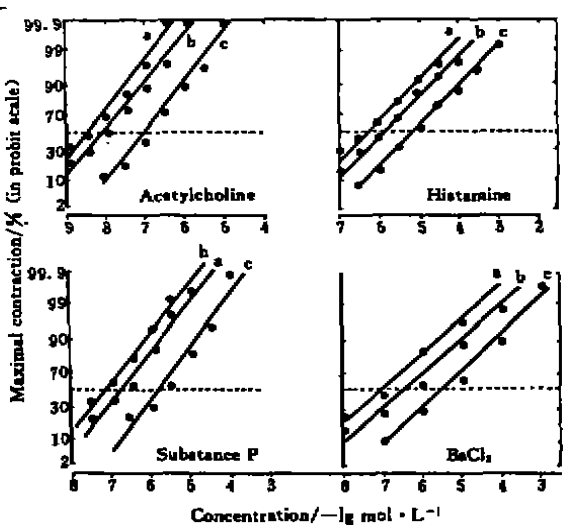

Fig 1. Contraction of sensitized guinea pig tracheal preparations *in vitro* after administration of acetylcholine, histamine, substance P, and $BaCl_2$. a: Epithelium removed mechanically; b: Pre-treated with antigen 48 h previously; c: Control. $n=8$.

EC_{50} of the constrictor agonists obtained from ERP decreased to 1/6—1/30 *vs* the control (Tab 1).

Tab 1. Contraction EC_{50} (nmol · L^{-1}) of drugs obtained in sensitized guinea pig tracheal preparation *in vitro*. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Drugs	Control	Pre-treated with antigen	Epithelium removed mechanically
ACh	128±63	10±3^c	40±1^c
His	5 900±941	901±241^c	260±50^c
SP	2 089±71	71±18^c	158±73^c
$BaCl_2$	4 224±1 142	277±69^c	78±14^c

Antigen-antibody reaction (adding 25 % ovalbumin 5 μl into 5 ml Krebs solution) to the sensitized guinea pig tracheal preparations induced a quick contraction lasting over 30 min. The contractile amplitudes of ERP were 50 %—100 % greater than those of the control (Tab 2).

Tab 2. Contractions (mg) of sensitized guinea pig tracheal preparations induced by antigen-antibody reaction (AAR) and electric field stimulation (EFS) *in vitro*. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Groups	Control	Pre-treated with antigen	Epithelium removed mechanically
AAR	419±198	714±302^c	888±328^c
EFS	368±162	868±229^c	822±272^c

After electric field stimulation, the tension of the sensitized guinea pig tracheal preparation was at first increased and then decreased rapidly, forming a sharp contraction peak. The contraction amplitudes of ERP were over 100 % greated than those of the control ($n=8$, $P<0.01$, Tab 2).

DISCUSSION

The present results showed that the reactivity of sensitized guinea pig tracheal smooth muscle with epithelial cells removed by mechanical method or antigen challenge to ACh, His, SP, and $BaCl_2$ was much greater than that of the control, indicating that antigen challenge could induce a severe damage or loss of tracheal epithelial cells, and that the existence of the epithelium inhibited the contractive effects of contrictor agonists on airway smooth muscle. The contraction amplitudes of ERP induced by antigen-antibody reaction increased 50 %— 100 % *vs* the control. These results suggested that epithelium removal not only induced an increase in reactivity of airway smooth muscle to constrictor agonists, but also play an important role in the regulation of allergic airway smooth muscle contraction. It strongly supported the hypothesis that non-specific hyperreactivity of asthma might be related to the removal of airway epithelium[5].

In our present experiment, the contraction amplitudes of epithelium-removed tracheal smooth muscle induced by EFS were over 2 times greater than those of the control. This result was very difficult to explain with the barrier theory[4], because the contractive responses induced by EFS were of neurogenic origen. The hyperreactivity of ERP might be related to removal of the effect of epithelium-derived relaxing factor[8].

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去上皮对离体气管平滑肌反应性的影响

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摘要 去上皮致敏豚鼠气管标本对乙酰胆碱、组胺，P物质和氯化钡的敏感性比未去上皮标本为高。上述药物在去上皮标本上得到的EC_{50}仅为未去上皮的1/6—1/30。由抗原抗体反应或电场刺激所引起的去上皮标本的收缩幅度和对照组相比升高了50 %—100 %。这些实验结果提示，气管上皮对呼吸道，尤其是对其过敏性收缩具有重要调节作用。

关键词 乙酰胆碱；组胺；P物质；氯；气管；上皮；抗原-抗体反应