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Original Research

Responses to adenosine of isolated transverse or spiral strips of sensitized guinea pig trachea: role of epithelium

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KEY WORDS adenosine; guinea pigs; trachea; epithelium; ovalbumin; dipyridamole

ABSTRACT

AIM: To determine the role of the epithelium in the responses to adenosine of isolated trachea from ovalbumin-sensitized guinea pigs. **METHODS**: Spirally cut tracheal preparations were superfused or immersed in organ baths and transversely cut strips were immersed. Epithelium was removed mechanically from some strips and confirmed by histological examination of a random sample. Tissues were from unsensitized or ovalbumin-sensitized guinea pigs. Isometric tension was measured and responses to adenosine recorded. **RESULTS**: In sensitized tissues, contractile responses to adenosine were evident as contractions of superfused spirals or as rightwards shift of the concentration-response curve compared with non-sensitized immersed spirals. Epithelium removal potentiated relaxation responses in both non-sensitized and sensitized strips indicating release of contractile mediators in both tissues. Dipyridamole potentiated relaxation responses in sensitized tissues with and without epithelium. **CONCLUSION**: Sensitization reveals a contractile response to adenosine. The epithelium is not involved in this contractile response nor is it the major site of uptake of adenosine in both sensitized and non-sensitized tissues.

INTRODUCTION

The loss of, or damage to, the epithelial layer has been shown to alter the responsiveness of airway smooth muscle preparations to a variety of agents. These effects have been related to the epithelium serving as a diffusion barrier[1], as an uptake site[2], or a source of epithelium-derived relaxant factor (EpDRF)[3]. Removal of the epithelium for example by ozone exposure causes airway hyperreactivity to spasmogens[3]. The epithelium-derived relaxant factor is probably nitric oxide since prevention of its formation also causes airway hyperreactivity[4]. Deficiency of endogenous nitric oxide generated via constitutively expressed or inducible nitric oxide synthases (iNOS) in the epithelium or other sources in the airways can explain the airway hyperreactivity after viral infection[5] or allergen challenge of sensitized guinea pigs[6].

Adenosine administered to the immersed isolated trachea usually induces a dose-dependent relaxation[7,8] occasionally preceded by a small transient contraction[1,9]. Removal of the epithelium has been reported to increase the sensitivity of the isolated trachea to the relaxant response to adenosine. This was proposed to be due to the epithelium acting either as a metabolic-uptake sink[10] or as the source of an excitatory substance[2]. We have shown that the contractile component of the response to adenosine is enhanced in tracheal preparations and airway perfused lungs taken from guinea pigs sensitised to ovalbumin[11-13]. This is comparable to the asthmatic or atopic patient who responds to inhaled adenosine with bronchoconstriction, compared with no response in normal subjects[14,15].

Loss or damage to the epithelial layer is a distinctive feature of asthma[16] and may contribute to the airway hyperreactivity of asthmatic subjects[17]. This study therefore investigates the effects of adenosine in isolated tracheal strips from both untreated and sensitized animals in an attempt to determine the role of the epithelium in the relaxation and contractile responses. Since the method of cutting the tracheal strips has been shown to affect the demonstration of relaxation or contractile responses to

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adenosine[18], we set up both immersed spirally cut tracheal strips and immersed transversely cut tracheal strips. The latter were more convenient for examining the removal of epithelium. We also compared immersed and superfused spirally cut tracheal preparations. The role of the epithelium as an uptake sink for adenosine was further examined by use of the adenosine transport inhibitor, dipyridamole in both epithelium intact and denuded strips from sensitized animals.

MATERIALS AND METHODS

Animals Male Dunkin-Hartley guinea pigs (Halls, Staffordshire, UK) weighing 300 – 400 g at purchase were housed at (22 ± 1) ℃ under 12 h normal phase light-dark cycle. They were fed on Special Rabbit Pellet (plain) 680 (Grain Harvesters, Canterbury, Kent, UK). Drinking water supplemented with ascorbic acid was provided *ad libitum*. Animals were killed by cervical dislocation, the tracheae excised and placed in Krebs-bicarbonate solution of composition (mmol/L): NaCl 118, $NaHCO_3$ 24.9, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, KH_2PO_4 1.15, and glucose 5.5.

Superfused tracheal spirals The trachea was cut spirally (2 mm wide) and lengths (3 – 4 cm) were suspended in a heated jacket (37 ℃). They were superfused with warmed gassed (5 % CO_2 in oxygen) Krebs-bicarbonate solution at a constant flow rate of 5 mL/min. One end was attached to a tissue holder and the other to a Devices UF1 isometric transducer (57 g sensitivity range).

Transverse tracheal strips Following the removal of adhering fat and connective tissue, the trachea was slit open along its longitudinal axis, directly opposite the smooth muscle. Two adjacent transverse strips consisting of three cartilage rings were prepared. For those strips where the epithelium was to be removed, the luminal surface (both smooth muscle and cartilage) was gently rubbed with a cotton-tipped applicator[19].

The strips were then set up, with one cartilage end attached to the aerator-tissue holder, the other to a Devices UF1 isometric transducer and immersed in warmed (37 ℃) Krebs-bicarbonate solution gassed with 5 % CO_2 and 95 % O_2 in 15 mL organ baths. Some experiments were also performed with tracheal spirals immersed in the same way.

In both tracheal spirals and strips, intrinsic tone was allowed to develop by leaving the strips to equilibrate under an applied tension of 1 g for 60 min. Changes in tension were recorded on a Devices M19 polygraph (Lectromed, Welwyn Garden City, Hertfordshire, UK).

Experimental protocols In superfused spirals, adenosine was added as 0.1 mL bolus doses injected into the perfusion solution immediately prior to entry to the warming coil. Dose-response curves were constructed by half-logarithmic increments in dose, each dose added when the tension had returned to the resting level after the changes from the previous dose. In immersed spirals or strips, cumulative concentration-response curves were constructed to adenosine, increasing concentration at half-logarithmic increments. After the final response, a supramaximal concentration of isoproterenol (isoprenaline) 14 μmol/L was added. The responses to adenosine were then expressed as a percentage of this maximum.

The tissues were used as pairs, either one being denuded of the epithelium, and the other remaining intact or one in the absence and the other in the presence of dipyridamole (2 μmol/L) which was preincubated for 30 min. The tissues were removed from untreated or ovalbumin-sensitized guinea pigs. Sensitization with ovalbumin was performed by administration of ovalbumin by intraperitoneal (ip) injections at 14 d (5 mg) and 12 d (10 mg) before use. The ovalbumin was dissolved in water for injection (150 mg in 3 mL)[20].

Analysis of results Responses were measured at each concentration or dose of adenosine as the peak change in tension from the resting level. These were then expressed as percentage ($\bar{x} \pm s_{\bar{x}}$) of the response to a supramaximal dose of isoproterenol.

Concentration-response curves were plotted for individual tissues and the EC_{30} or EC_{50} measured as the molar concentration for a 30 % or 50 % of the isoproterenol maximum response by linear interpolation between points on either side. Geometric mean EC_{30} or EC_{50} values were then calculated together with their 95 % confidence limits.

Significance of difference was calculated by paired or unpaired, two-tailed *t*-tests between responses from the same or different animals, respectively. Significance was assumed at the 5 % probability level.

Histology Four pairs of tissues were selected for histological examination. Sections of intact and denuded tracheae, 10 – 30 μm thick, were prepared, stained with haematoxylin and eosin and examined microscopically for the presence or absence of epithelium.

Drugs Adenosine, dipyridamole, and (-)-isoproterenol bitartrate were obtained from Sigma (Dorset,

UK) and ovalbumin from BDH (Dorset, UK). Adenosine and isoproterenol were dissolved in 0.9 % saline (normal saline) and dipyridamole was initially dissolved in 0.1 mL of HCl (1 mol/L) before further dilution with normal saline. Solutions of isoproterenol contained ascorbic acid (3 mmol/L) as an antioxidant.

RESULTS

Comparison of superfused and immersed spirals In unsensitized superfused tracheal spirals, adenosine caused dose-related relaxation responses only. In sensitized preparations, there was a small contractile response at lower doses, which converted to a relaxation at higher doses (Fig 1A). In immersed tracheal spirals from sensitized animals with intact epithelium, the constrictor phase was absent from the concentration-response curves (Fig 1B). However, the concentration-response curve for the relaxation response in the sensitized tissue was to the right of that in the non-sensitized trachea. The relaxation responses at the intermediated doses were significantly less in the sensitized tissues ($P<0.05$).

Epithelium removal-histology Examination of unrubbed tracheae using a light microscope confirmed the presence of the epithelium and individual cilia were clearly seen (Fig 2A). Conversely, in rubbed tissue, the epithelium was completely removed (Fig 2B). Both photographs in Fig 2 clearly showed smooth muscle and cartilage.

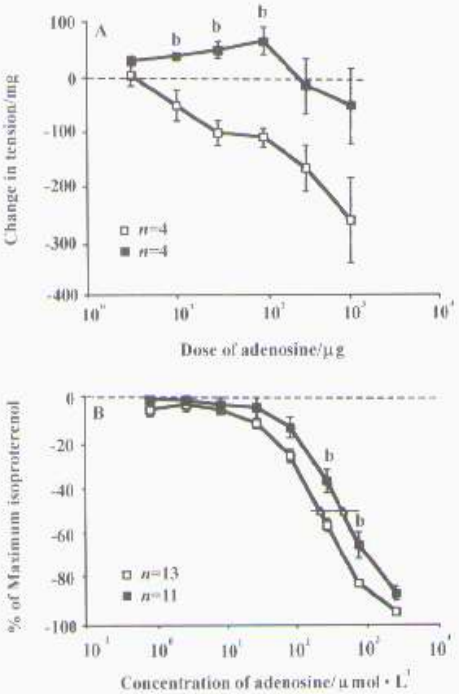

Fig 1. Effect of adenosine on A) superfused and B) immersed tracheal spirals from unsensitized (□) or ovalbumin-sensitized (■) guinea pigs. Geometric mean EC_{50} values are shown by △. $\bar{x} \pm s_{\bar{x}}$. $^bP<0.05$ *vs* control tissues.

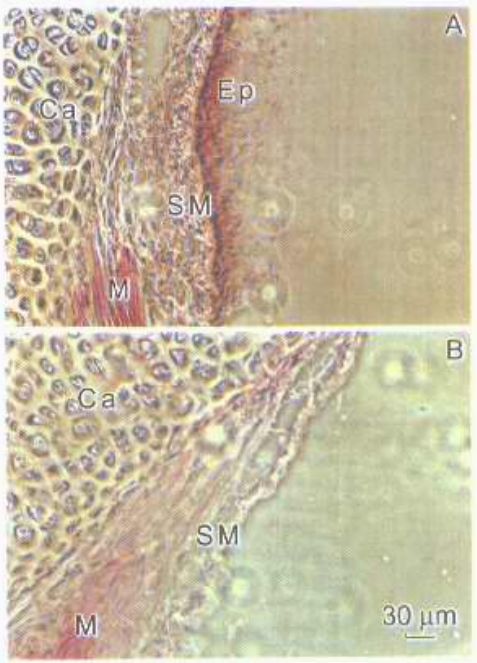

Fig 2. Micrographs of sections of guinea pig tracheal strips, stained with haematoxylin and eosin, showing intact (A) and denuded (B) epithelium under phase contrast. × 300. Bronchial smooth muscle (M), cartilage (Ca), and the submucosa (SM) may be seen in both, but ciliated columnar epithelium (Ep) is only present in the intact tissue.

Epithelium removal-effect on adenosine responses in unsensitized tracheal strips Adenosine induced concentration-dependent relaxations in both rubbed and control (epithelium intact) immersed tracheal strips (Fig 3). In denuded preparations, the higher doses induced significantly greater relaxations ($P<0.05$, Fig 3A).

Effects of ovalbumin-sensitization on tracheal strips In tracheal strips from sensitized guinea pigs, adenosine induced concentration-dependent relaxations (Fig 3B) without initial contractions at low concentra-

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tions. The concentration-response curve for the denuded preparation fell to the left of the intact tissue (Fig 3B) with the relaxation responses at the higher doses significantly greater than in the intact tracheas ($P<0.05$).

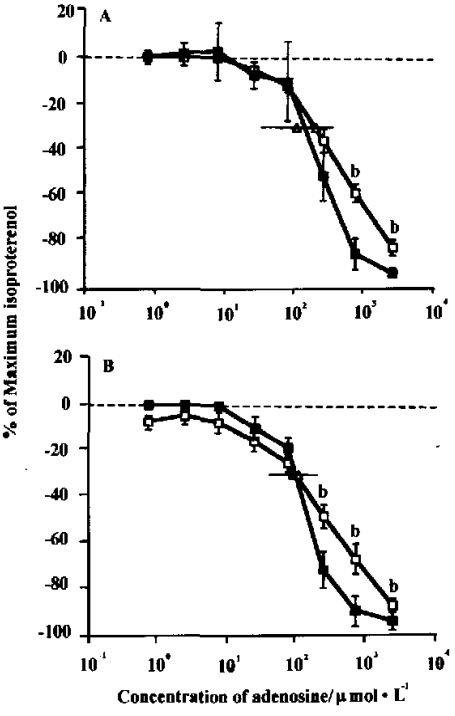

Fig 3. Effects of adenosine on epithelium intact (□) and epithelium-denuded (■) paired immersed tracheal strips from A) unsensitized ($n=5$) and B) ovalbumin-sensitized guinea pigs ($n=6$). Geometric mean EC_{30} values are shown by △. $\bar{x} \pm s_{\bar{x}}$. $^bP<0.05$ *vs* epithelium-denuded tissues.

Both the final isoproterenol response and the initial baseline tone in sensitized tissue were significantly lower in the denuded tissue compared with the intact tissue ($P<0.05$, Fig 4). No differences were observed between unsensitized and intact sensitized resting tone and isoproterenol-induced relaxation.

Comparision of immersed tracheal strips from untreated and ovalbumin-sensitized guinea pigs, showed little difference in either intact (Fig 5A) or denuded tracheae (Fig 5B).

Effect of dipyridamole upon responses to adenosine in intact and denuded tracheal strips from sensitized guinea pigs In the presence of the adenosine transport inhibitor, dipyridamole (2 μmol/L),

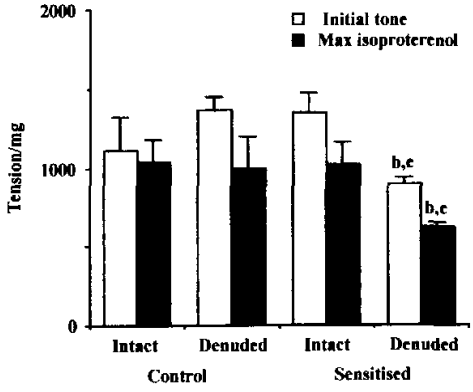

Fig 4. Comparison of initial baseline tone and maximum relaxation responses to isoproterenol 5.4 μmol/L in tracheal strips, with the epithelial layer intact or mechanically denuded. Tissues were obtained from unsensitized (Control, $n=5$) and ovalbumin-sensitized guinea pigs ($n=6$). $\bar{x} \pm s_{\bar{x}}$. $^bP<0.05$ *vs* intact tissues. $^cP<0.05$ *vs* control.

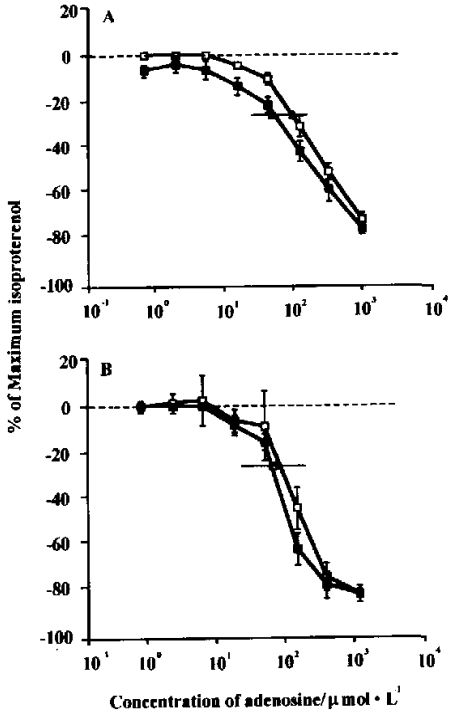

Fig 5. Effects of adenosine on tracheal strips from unsensitized (□, $n=5$) and ovalbumin-sensitized guinea pigs (■, $n=6$) in which the epithelium was A) intact or B) mechanically removed. Geometric mean EC_{30} values are shown by △. $\bar{x} \pm s_{\bar{x}}$.

the submaximal responses to adenosine of sensitized tracheal strips were potentiated in both intact and denuded preparations (Fig 6). The curves were significantly shifted to the left by 20 folds in both intact and denuded tissues ($P < 0.05$). Similarly, for both preparations, the maximum relaxation induced by adenosine in the presence of dipyridamole was significantly reduced ($P < 0.05$).

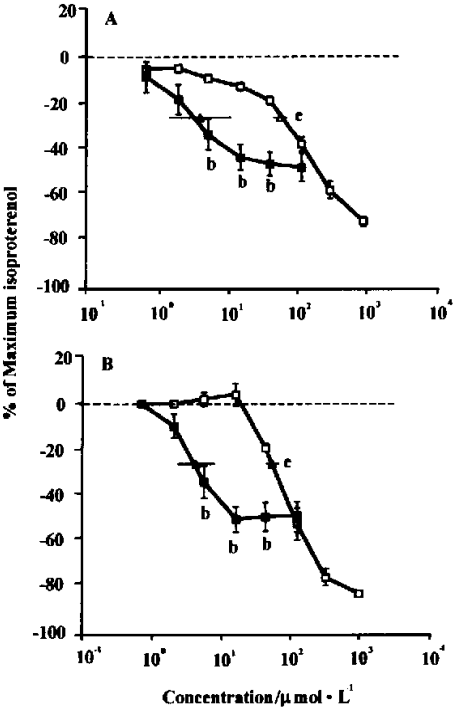

Fig 6. Effects of dipyridamole on the responses of tracheal strips from ovalbumin-sensitized guinea pigs to adenosine. Concentration-response curves for adenosine were obtained in the absence (control, □, $n = 5$) or presence of dipyridamole 2 μmol/L (■, $n = 5$) in tracheal strips in which the epithelium was A) intact or B) mechanically removed. Geometric mean EC_{30} values are shown by △. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$ *vs* control. $^cP < 0.05$ *vs* EC_{30} values of dipyridamole-treated group.

DISCUSSION

The predominant response of isolated guinea pig tracheal preparations to adenosine is relaxation[7,21]. Occasionally, however, contractions have also been described[8,18,22,23]. The contraction is usually observed in response to the lower doses and, normally, only in preparations which have been allowed to equilibrate under intrinsic tone. Contractions were also favoured in spirally cut tracheal preparations compared with transversely cut preparations[18]. In the present study, no contractions occurred in unsensitized preparations of either spirally cut or transversely cut tissues. The predominant relaxation is consistently induced at higher doses and at any level of resting tone, intrinsic or precontracted. Holroyde[1] reported that contractions could only be induced in epithelium-denuded preparations. In the present study denuding of the epithelium did not reveal a contraction in unsensitized tissues. However, the relaxation responses were significantly greater after epithelium denuding and this would suggest the removal of an underlying constrictor component which would normally oppose the predominant relaxant response. Indeed, Farmer *et al*[2] suggested that an excitatory (contractile) substance was released from the epithelium by adenosine. The possible nature of such a contractile agent is prostaglandin $F_{2\alpha}$ or a leukotriene[17]. There was no evidence of epithelium-derived relaxant factors (EpDRF's), such as PGE_2 and nitric oxide, that are proposed to be released by other broncho-active agents[17,24-26]. Removal of such factors by denuding the epithelium would have reduced the relaxation response, not enhanced it.

In confirmation of our previous observations[11-13], adenosine produced a contractile response at lower doses in the superfused tracheal spiral from sensitized guinea pigs. This contrasts with the relaxation responses seen in unsensitized tissue. In the immersed preparations, however, there was no contractile phase of the concentration-response curve. In the immersed tracheal spiral, the concentration-response curve for sensitized tissue was to the right of that for the unsensitized tissue. This suggests that the underlying constrictor component to the adenosine response in sensitized tissues is opposing the dominant relaxation response. In the immersed transverse tracheal strips, however, there was no difference between sensitized and non-sensitized tissues. These tissues would appear to display a highly dominant relaxation response in which the contractile response to adenosine of sensitized tissue is overwhelmed. This is probably due to the additive effects of transverse cutting of the tissue which does not appear to favour the contractile elements[18] and immersion rather than superfusion. If the contractile responses are due to release of mediators[17], then they may readily diffuse into the bathing fluid and be diluted before they can exert their full effects. In superfused tissues, the local levels of mediators may remain

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relatively high before being washed away.

Epithelial removal in sensitized tissues had an identical effect on the adenosine concentration-response curve to that in non-sensitized tissues. The potentiation of the relaxation response indicated that there was an epithelium-derived contractile factor also in sensitized tissue but sensitization did not alter this. Thus, in sensitized tissues there was no additional shift of the curves to the left when the epithelium was removed, compared with that seen in unsensitized tissues. Therefore it was unlikely that the epithelium was the source of any further underlying contractile component. The contraction seen in the sensitized superfused tracheal spirals therefore had a separate origin to the epithelium-derived contractile factor that occurs in both sensitized and non-sensitized tissues.

The resting tone was reduced by epithelium removal only in sensitized tracheal strips and this was accompanied by a reduced relaxation response to the maximally effective concentration of isoproterenol. The resting tone of bronchial tissue, from as far back as 1975, has been speculated to result from the apparently spontaneous release of various mediators from within the tissue such as products of the cyclooxygenase cascade including $PGF_{2\alpha}$[27]. Subsequently, various groups have performed experiments in isolated respiratory preparations in the presence of indomethacin to minimise this resting tone[28-30]. The *in vitro* response of guinea pig trachea to exogenous arachidonic acid was converted from a relaxation to a contraction in the presence of indomethacin. Removal of the epithelium had a similar effect, implying epithelial involvement in prostaglandin synthesis[17,31]. However, in a similar preparation to that used in the present study, Lundblad and Persson[32] concluded that mechanical removal of the epithelium apparently has little influence upon the development of spontaneous tone in the guinea pig isolated trachea. This is consistent with the results obtained in the present study using unsensitized tissue. Only in the sensitized tissues did epithelial removal reduce resting tone suggesting that the epithelium was responsible for some resting tone in sensitized tissues only.

To determine the role of the epithelium as an uptake site for removal of adenosine, the transport inhibitor, dipyridamole, was examined in sensitized tracheal strips. In confirmation of its effects in previous studies in non-sensitized epithelium-intact tracheal preparations[18,21], dipyridamole potentiated the adenosine relaxation responses. There is therefore an efficient system for removal of adenosine and when this is inhibited by dipyridamole, more extracellular adenosine is available for interaction with the extracellular A_2 receptors mediating relaxation[21]. The attenuation of the maximum response to adenosine observed in the present study has been ascribed to the removal of an intracellular P-site by dipyridamole[21]. Denuded sensitized tracheae behaved in a similar fashion with a similar depression of the maximum and a comparable shift to the left, in the presence of dipyridamole. Similar effects were found by Advenier *et al*[10] who observed a potentiation of the relaxant effects of adenosine in non-sensitized guinea pig trachea with intact epithelium in the presence of dipyridamole. In the denuded preparation, they found dipyridamole still potentiated adenosine but had no effect additional to epithelial removal. They concluded that the epithelium was an uptake and metabolism site for adenosine, but this conclusion was not supported by the present study. Since dipyridamole still potentiated the response to adenosine in the absence of the epithelium, uptake and metabolism must, therefore, occur at another site. Further studies should include alternative inhibitors of adenosine transport, such as *p*-nitrobenzylthioinosine (NBTI), to confirm these observations.

In conclusion, this study has shown that the epithelium has a minimum role in the responses of isolated trachea to adenosine. There is no evidence for the epithelium operating as a site of adenosine uptake or of adenosine-induced release of relaxant mediators. There was evidence of the epithelium being a site of release of a contractile mediator by adenosine in both non-sensitized and sensitized trachea. This is not the source of the contractile response seen only in sensitized superfused tracheal spirals. Thus, the pathological observation of epithelial loss in asthma[16] would not be expected to affect the bronchoconstriction by adenosine in asthmatics[14,15]. The bronchoconstriction would appear to be independent of the epithelium. Further studies using superfused tissues and bronchioles from sensitized guinea pigs could provide additional insight into this issue.

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致敏离体豚鼠气管横向或螺旋条对腺苷的收缩反应:上皮的作用

关键词 腺苷; 豚鼠; 气管; 上皮; 卵白蛋白; 双嘧达莫

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