Br. J. Pharmacol. (1988), **93**, 295–302

Influence of epithelium on the responsiveness of guinea-pig isolated trachea to adenosine

[1]Charles Advenier, *Philippe Devillier, *Régis Matran & Emmanuel Naline

Faculté de Médecine Paris-Ouest, Laboratoire de Pharmacologie, 15, rue de l'Ecole de Médecine, 75270 Paris Cedex 06 and *Services de Pneumologie (Pr J. Marsac) et d'Explorations Fonctionnelles Respiratoires (Pr A. Lockhart), Hôpital Cochin, 27, rue du Fbg Saint-Jacques, 75014 Paris, France

1 The influence of epithelium removal on the effects of adenosine on airway contractility was investigated on the guinea-pig isolated trachea.

2 In preparations under resting tone or precontracted with histamine 10^{-5} M, removal of the tracheal epithelium resulted in similar shifts to the left of the adenosine concentration-response curves (0.61 ± 0.18 ($P < 0.05$) and 0.80 ± 0.09 ($P < 0.001$) log units; $n = 5$), corresponding to 4.07 and 6.31 fold potentiations of the relaxant effect of adenosine.

3 In the presence of dipyridamole 10^{-5} M the relaxant effects of adenosine were potentiated 85.1 fold on tracheae with epithelium; removal of the epithelium did not produce a significant additional shift to the left of the adenosine concentration-response curves (0.07 ± 0.03 log units; $n = 5$; NS).

4 In the absence of dipyridamole, the theophylline-adenosine antagonism was not of the competitive type, irrespective of whether the tracheae were with or without epithelium.

5 In the presence of dipyridamole, this antagonism was likely to be of the competitive type and its characteristics were the same when the epithelium was present or absent. Regression slope and pA_2 values were 0.84 and 5.07, respectively, in the presence of epithelium and 0.76 and 4.89, respectively, in its absence.

6 It is suggested that, at least in the guinea-pig isolated trachea model, the airway epithelium seems to be involved only in the uptake and metabolism of adenosine.

Introduction

The airway epithelium is known to exert an influence on bronchial smooth muscle reactivity. The mechanical removal of the epithelial layer increases the responsiveness of isolated tracheae from several species to a variety of bronchoconstrictor agents, such as histamine, acetylcholine, 5-hydroxytryptamine and substance P and to antigenic stimulation (Barnes *et al.*, 1985; Flavahan *et al.*, 1985; Goldie *et al.*, 1986; Frossard & Müller, 1986; Hay *et al.*, 1986a,b; Holroyde, 1986; Raeburn *et al.*, 1986; Tschirhart & Landry, 1986) and it has been suggested that epithelial cells may secrete an inhibitory factor which modulates airway smooth muscle tone (Barnes *et al.*, 1985; Flavahan *et al.*, 1985; Raeburn *et al.*, 1986; Tschirhart & Landry, 1986). The influence of epithelium on responsiveness to bronchial muscle relaxants is more complex. In experiments on guinea-pig tracheae deprived of their epithelium, the relaxant activity of isoprenaline was either increased (Holroyde, 1986; Farmer *et al.*, 1986; Hay *et al.*, 1986a,b) or unmodified (Goldie *et al.*, 1986), while that of sodium nitroprusside was increased and that of papaverine was unchanged (Farmer *et al.*, 1986). In similar experiments on isolated canine and bovine tracheae without epithelium, the activity of isoprenaline was reduced (Barnes *et al.*, 1985; Flavahan *et al.*, 1985).

Few studies have been devoted to the influence of epithelium on the responsiveness of airway smooth muscle to adenosine. Holroyde (1986) found that after removal of the epithelium the concentration-response curves to adenosine on guinea-pig isolated trachea were shifted to the left, indicating potentiation. Farmer *et al.* (1986) using the same *in vitro* model noted that potentiation only occurred in the presence of dipyridamole, which inhibits adenosine tissue uptake, and of erythro-9-2-hydroxy-3nonyladenine (EHNA), which inhibits adenosine desaminase.

In the present study, the influence of epithelium on

[1]Author for correspondence.

the relaxant effect of adenosine on airway smooth muscle was evaluated by measuring this effect and by investigating the adenosine-theophylline antagonism on guinea-pig isolated tracheae with or without epithelium and in the presence or absence of dipyridamole.

Methods

Tissue preparation

Male guinea-pigs (250–350 g) were killed by a blow to the head and exsanguinated. The trachea was removed and placed in Krebs-Henseleit solution (composition mM: NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.7). Following removal of adhering fat and connective tissue the trachea was slit open along its longitudinal axis, directly opposite the smooth muscle, and two strips consisting of 3 adjacent cartilage rings were prepared according to the zig-zag method of Emmerson & Mackay (1979). On one of these strips, the epithelium was removed by gently rubbing the luminal surface (over both the smooth muscle and cartilage areas) with a cotton-tipped applicator (Tschichart & Landry, 1986; Hay *et al.*, 1986a,b; Raeburn *et al.*, 1986b); the other strip served as a paired control.

The strips were then suspended in 25 ml organ baths containing Krebs-Henseleit solution at 37°C, gassed with 95% O_2 and 5% CO_2, and equilibrated under an initial tension of 1.50 g.

After equilibration for 1.25 h, the resting tension was between 0.6 and 1.4 g. Resting tension in strips with and without epithelium were not significantly different (958 ± 56 mg and 1008 ± 77 mg, respectively, $n = 39$, NS). Under these conditions, responses to agonists were reproducible. Tension was measured isometrically with a Gould strain gauge (UC 3) and was displayed on a Bryans BS 2H recorder.

Protocols

In all experiments, tracheal spirals were first contracted to maximal tension with carbachol 10^{-4} M. After 1 h rest, with washing every 15 min, cumulative concentration-response curves to adenosine were obtained by increasing the concentration of adenosine at 5–10 min intervals in logarithmic increments, the tracheal strips being either under resting tone or precontracted with histamine 10^{-5} M. After the concentration-response curve to adenosine was completed, theophylline 3×10^{-3} M was added to the bath to determine maximal relaxation. Histamine usually produced greater responses in strips without epithelium, but as the number of strips in each group was small, the differences between strips with and without epithelium were non significant. Tracheal strips were pretreated with dipyridamole (10^{-5} M) or theophylline (10^{-5} to 10^{-4} M) 10 min before addition of histamine. Owing to the development of tachyphylaxis, only one series of adenosine concentration was used for each tracheal preparation. The concentration of dipyridamole (10^{-5} M) was chosen from previous experiments (Advenier *et al.*, 1982) where concentration-effect curves to dipyridamole on the relaxant effects of adenosine were performed; in these experiments dipyridamole 10^{-5} M gave the greatest shift to the left of the concentration-responses to adenosine.

The adenosine-induced relaxation was expressed as a percentage of the maximal effect of theophylline 3×10^{-3} M. $-\log EC_{50}$ values (defined as the negative log of the drug concentration that caused 50% of maximal effect of theophylline 3×10^{-3} M), were derived from the log concentration-effect curves. These values were evaluated graphically from each experiment. pA_2 values were determined according to Arunlakshana & Schild (1959).

Control of epithelium removal

Epithelium removal was checked by histological studies in some cases and pharmacologically, by testing the relaxant effect of arachidonic acid (10 μM) on preparations contracted with carbachol 10^{-6} M. Relaxation responses to arachidonic acid were 44.4 ± 5.7% ($n = 15$) and 3.7 ± 1.6% ($n = 15$) respectively of the maximal relaxation induced by theophylline in preparations with or without epithelium.

Statistical analysis of results

Statistical analysis of the results obtained was performed using Student's t test. All values in the text and table are expressed as mean ± s.e.mean.

Drugs

The drugs used were: adenosine (Merck, D-Darmstadt), histamine (Sigma, St. Louis, U.S.A.), dipyridamole (Boehringer-Ingelheim, F-Reims), theophylline sodium anisate (Bruneau, F-Paris). Theophylline was used as proprietary injectable solution (Theophylline Bruneau); dipyridamole was dissolved daily in ethanol and the solution was further diluted with Krebs solution.

Results

Influence of epithelium and dipyridamole on adenosine concentration-response curves

Adenosine exerted a relaxant effect on the guinea-pig

Table 1 Influence of dipyridamole 10^{-5} M and of epithelium removal on $-\log EC_{50}$ of adenosine and on adenosine concentration-relaxant response curves in the guinea-pig isolated trachea

Pretreatment of preparation	*$-\log EC_{50}$ with epithelium*	*without epithelium*	*Shift of the C-R curves log unit*	*Significance*
None	3.43 ± 0.09	4.04 ± 0.13	0.61 ± 0.18	$P<0.05$
Histamine 10^{-5} M	3.40 ± 0.12	4.20 ± 0.15	0.80 ± 0.09	$P<0.001$
Histamine 10^{-5} M + dipyridamole 10^{-5} M	5.33 ± 0.20	5.40 ± 0.17	0.07 ± 0.03	NS

Experiments were performed on 5 preparations. Values are mean ± s.e.mean.

isolated trachea (Figures 1 and 2). In the absence of dipyridamole, this effect was the same on tracheae under resting tone and on tracheae precontracted with histamine 10^{-5} M, the $-\log EC_{50}$ values of adenosine being 3.43 ± 0.09 ($n = 5$) and 3.40 ± 0.12 ($n = 5$) respectively (Table 1).

Removal of the epithelium resulted in significant shifts to the left of the adenosine concentration-response curves (Figures 1 and 2), irrespective of whether the tracheae were under resting tone or precontracted with histamine. The shifts were 0.61 ± 0.18 and 0.80 ± 0.09 log units ($n = 5$) respectively (Table 1).

In the presence of dipyridamole, the effect of adenosine on tracheae with epithelium was potentiated 85.1 fold, as shown by comparison with the $-\log EC_{50}$ for the effect of adenosine on histamine-precontracted tracheae. In epithelium-denuded strips, dipyridamole also potentiated (15.8 fold) the responses produced by adenosine but this potentiation was less than observed in strips with epithelium, so that, in the presence of dipyridamole, epithelium removal did not affect adenosine sensitivity (Figure 2, Table 1).

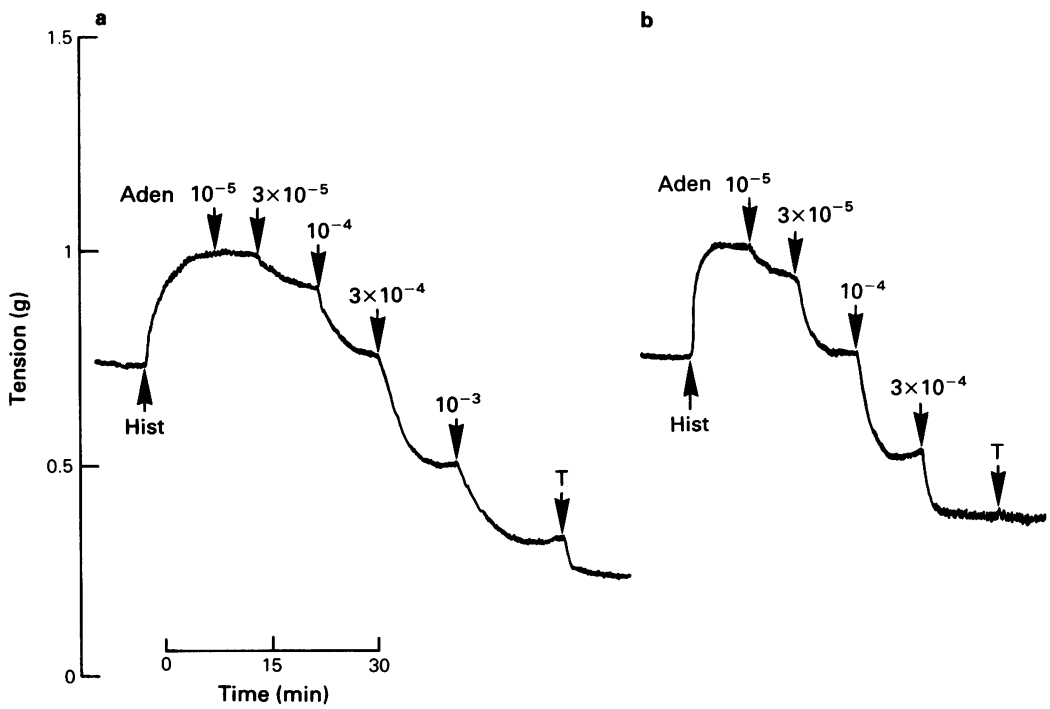

Figure 1 Example of cumulative adenosine (Aden) concentration-response curve in the presence (a) or absence (b) of epithelium in the guinea-pig isolated trachea. T is theophylline 3×10^{-3} M. Hist is histamine 10^{-5} M.

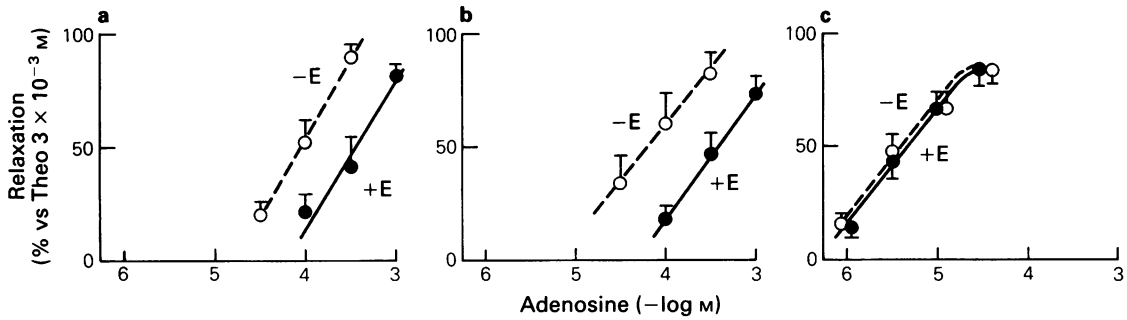

Figure 2 Influence of epithelium removal on adenosine concentration-response curves on the guinea-pig isolated trachea under resting tone (a) or precontracted with histamine 10^{-5} M in the absence (b) or in the presence of dipyridamole 10^{-5} M (c). Effects of adenosine are represented in the presence (●) (+ E) or in the absence (○) (− E) of epithelium. Vertical bars are s.e.mean from 5 experiments.

Table 2 Maximal relaxation induced by adenosine (mg)

Pretreatment	n	*With epithelium*	*Without epithelium*
None	5	ND	478 ± 124
Histamine 10^{-5} M	5	ND	944 ± 160
Histamine 10^{-5} M + dipyridamole 10^{-5} M	5	730 ± 173	770 ± 84
Histamine 10^{-5} M + theophylline 10^{-5} M	4	915 ± 135	987 ± 164
+ theophylline 3×10^{-5} M	4	817 ± 141	872 ± 148
+ theophylline 10^{-4} M	4	492 ± 103	460 ± 162
Histamine 10^{-5} M + dipyridamole 10^{-5} M			
+ theophylline 10^{-5} M	4	778 ± 84	843 ± 178
+ theophylline 3×10^{-5} M	4	744 ± 163	692 ± 307
+ theophylline 10^{-4} M	4	367 ± 95	455 ± 180

Values are mean ± s.e.mean. No significant differences were observed between preparations with and without epithelium. *n* = number of experiments. ND = not done.

Finally, adenosine exerted similar maximal relaxant effects on tracheae with or without epithelium, in the presence or absence of dipyridamole (Table 2).

Influence of epithelium and dipyridamole on adenosine-theophylline antagonism

Figure 3 shows the displacement, under the influence of increasing concentrations of theophylline, of the adenosine concentration-response curves on preparations with or without epithelium and in the presence or absence of dipyridamole. The values of regression slopes and pA_2 calculated by the method of Arunlakshana & Schild (1959) are given in Table 3.

It appears from these values that when the epithelium was present, theophylline 10^{-5} to 10^{-4} M had no antagonistic activity against adenosine in preparations without dipyridamole, whereas a competitive antagonism is suggested in the presence of dipyridamole 10^{-5} M, the pA_2 of theophylline then being 5.07.

On preparations without epithelium and in the absence of dipyridamole, the adenosine concentration-response curves were displaced to the right under the influence of theophylline in concentrations ranging from 10^{-5} M to 3×10^{-5} M. With these concentrations, a competitive antagonism appeared, with a regression slope of 0.94 and a pA_2 of 4.80. In contrast,

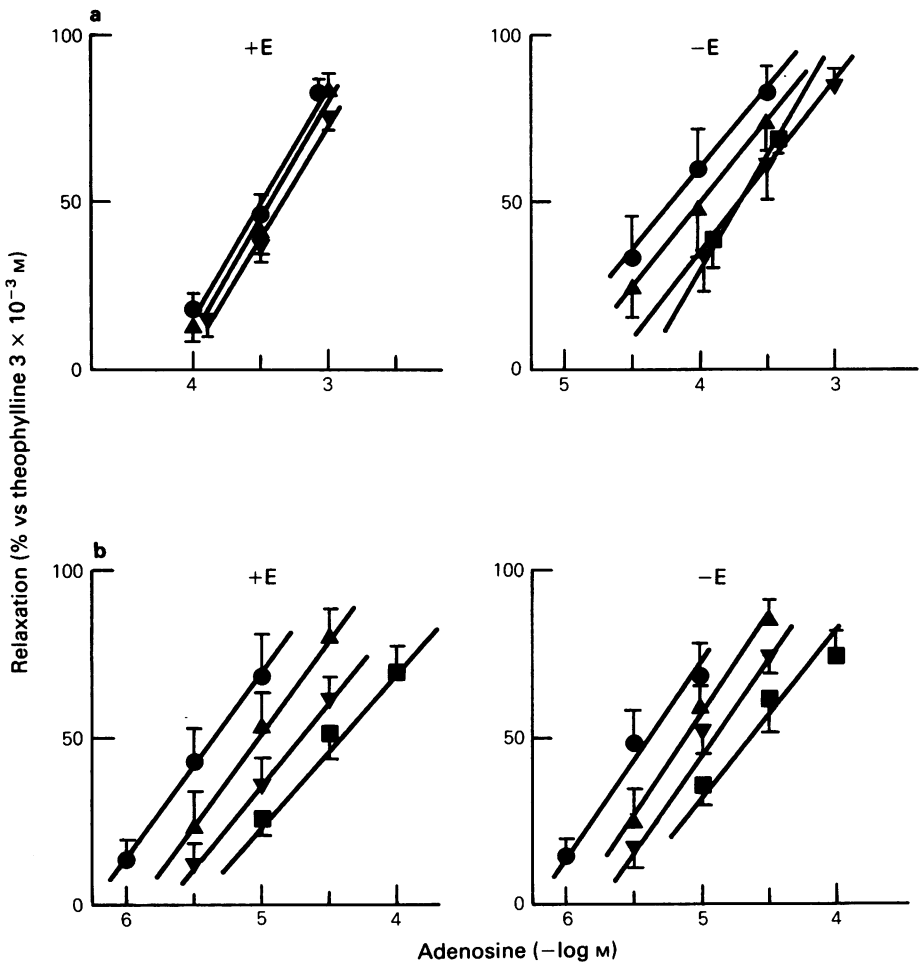

Figure 3 Theophylline-induced changes in the adenosine concentration-response curves on the guinea-pig isolated trachea in the presence (+E) or in the absence (−E) of epithelium and without (a) or with dipyridamole (b) (10^{-5} M). (●) Control; (▲) theophylline 10^{-5} M; (▼) theophylline 3×10^{-5} M; (■) theophylline 10^{-4} M. Vertical bars are s.e.mean from 5 experiments.

Table 3 Influence of epithelium removal and of dipyridamole on the characteristics of the antagonism between adenosine and theophylline

	With epithelium		*Without epithelium*		
	Without dipyridamole	*With dipyridamole* (10^{-5} M)	*Without dipyridamole* (*) (a)	(b)	*With dipyridamole* (10^{-5} M)
Slope of regression line	—	0.84	0.94	0.47	0.76
pA_2	—	5.07	4.80	—	4.89

(*) With concentrations of theophylline of (a) 10^{-5} M and 3×10^{-5} M; (b) 10^{-5} to 10^{-4} M.
Values were calculated from the mean dose-response curves obtained in Figure 3.

with theophylline 10^{-4} M, the adenosine concentration-response curves were no longer shifted to the right and the antagonism when theophylline concentrations of 10^{-5} to 10^{-4} M were used was not competitive (regression slope 0.47).

In the absence of epithelium and in the presence of dipyridamole, the adenosine-theophylline antagonism is likely to be competitive and had the same characteristics as those observed in the presence of both epithelium and dipyridamole.

Discussion

Adenosine probably plays an important part in the regulation of bronchomotility (Church *et al.*, 1986), but it seems to exert a dual effect, either contracting or relaxing the bronchial smooth muscle. Thus, Cushley *et al.* (1983, 1984) have demonstrated that adenosine behaves as a potent bronchoconstrictor agent when administered by inhalation to asthmatic patients, and this effect is inhibited by theophylline (Mann & Holgate, 1985). Adenosine also contracts the guinea-pig isolated trachea, but this effect is modest (Fredholm *et al.*, 1979; Karlsson *et al.*, 1982; Advenier *et al.*, 1982; Holroyde, 1986), and this substance has mainly a relaxant effect on this preparation (Coleman & Levy, 1974; Coleman, 1976; Farmer & Farrar, 1976; Christie & Satchell, 1980; Karlsson *et al.*, 1982; Advenier *et al.*, 1982), which implies that adenosine receptors are of the P_1 type (Burnstock, 1978; Christie & Satchell, 1980) or of the A_2/Ra type (Brown & Collis, 1982). The relaxant effect of adenosine is potentiated by dipyridamole (Coleman, 1976; Karlsson *et al.*, 1982; Advenier *et al.*, 1982), a compound that inhibits adenosine uptake (Kolassa *et al.*, 1970).

In our experiments, removal of the epithelial layer potentiated the effects of adenosine on guinea-pig tracheae which had not been pretreated with dipyridamole. The shift to the left of the adenosine concentration-response curves was the same on tracheae under resting tone (0.61 ± 0.18 log units) and on tracheae precontracted with histamine 10^{-5} M (0.80 ± 0.09 log units). In contrast, on tracheae pretreated with dipyridamole 10^{-5} M, the adenosine concentration-response curves were markedly displaced to the left (1.93 log units) when the epithelium was present, showing an 85.1 fold increase in adenosine potency. The adenosine concentration-response curves were also displaced to the left for tracheae without epithelium, but only to the same extent as for tracheae with epithelium; there was no significant additional shift (0.07 ± 0.03 log unit, NS). The results of our experiments on tracheae pretreated with dipyridamole were similar to those of Holroyde (1986), who observed a 0.50 log unit shift of adenosine concentration-response curves for guinea-pig tracheae under resting tone or under a 1 g tension, but they clearly differ from the results obtained by Farmer *et al.* (1986) working on tracheae prepared in the same way as those used by Holroyde (1986). According to Farmer *et al.* (1986), the adenosine concentration-response curves were not displaced in the absence of dipyridamole, but only when the preparations were pretreated with both dipyridamole and EHNA; however, these authors used dipyridamole in very low concentrations (3×10^{-7} M as against 10^{-5} M in our experiments), which may account for the discrepancy in results.

Concerning the part played by epithelium in the adenosine-theophylline interaction, our results clearly showed that on tracheae with epithelium but not pretreated with dipyridamole (Figure 1a) theophylline did not modify the effects of adenosine, as has been suggested by Karlsson *et al.* (1982). In contrast, in the presence of dipyridamole (Figure 2a) it seems that theophylline antagonizes adenosine in a competitive manner, with a regression slope of 0.84 and a pA_2 value of 5.07. Karlsson *et al.* (1982), working under conditions similar to ours (i.e. dipyridamole concentration 2×10^{-6} M and tracheae under resting tone) also found competitive antagonism with regression slope (1.18) and pA_2 (4.92) values almost identical to ours. It will be noted that theophylline seems to be much more active as an antagonist of adenosine than as a smooth muscle relaxant, since the pD_2 of theophylline against histamine- or acetylcholine-induced contraction of the guinea-pig isolated trachea is about 3 or 4 (Karlsson *et al.*, 1982; Advenier *et al.*, 1982).

Our study showed that when both epithelium and dipyridamole were absent, the shift in adenosine concentration-effect curves was similar to that observed in the presence of dipyridamole, but only with theophylline concentrations of 10^{-5} and 3×10^{-5} M; with concentrations of 10^{-4} M the displacement plateaued, which meant that the antagonism was not competitive. In contrast, when dipyridamole was present the antagonism appeared to be competitive, with the same characteristics as those observed in the presence of epithelium. These results suggest that degradation or uptake processes can strongly modify the study of the interaction between a ligand and its receptor (Kenakin, 1984).

In the light of these results, the role played by epithelium in the effects of adenosine on the guinea-pig isolated trachea preparation may be regarded as an essentially metabolic one.

(1) It could be argued that adenosine induces the release of a factor (Farmer *et al.*, 1986), analogous to but opposite in effect to the relaxant factor released under the influence of various contractive agents. The absence of release of this constricting factor in preparations deprived of epithelium would account for the shift to the left of the adenosine concentration-

response curves. However, this possibility is ruled out by the fact that in the presence of dipyridamole these curves are exactly the same as when the epithelium is absent or present.

(2) The epithelium may conceivably act as a barrier, as Holroyde (1986) suggested to explain the influence of epithelium on the effects of different substances, in which case removal of the epithelium would facilitate adenosine diffusion and displace the adenosine concentration-response curves; but this hypothesis, like the previous one, is untenable for the reason given above.

Our results appear to show that the airway epithelium merely intervenes in the uptake and/or degradation of adenosine. But they also show that other structures, such as airway connective tissue or smooth muscle, are also involved in adenosine metabolism, since in the absence of both epithelium and dipyridamole the adenosine concentration-reponse curves are only moderately shifted to the left, and since the antagonistic effect of theophylline to adenosine is not of the competitive type. Another consequence of our results is that the adenosine-theophylline interaction must be investigated in the presence of dipyridamole, and that whether the epithelium is absent or present is of little importance to this investigation.

This work was supported by a grant from the Scientific Concil of the Faculty of Medecine Paris-Ouest. The authors thank Dr G. Roux for his English translation.

References

ADVENIER, C., BIDET, D., FLOCH-SAINT-AUBIN, A. & RENIER, A. (1982). Contribution of prostaglandins and thromboxanes to the adenosine and ATP-induced contraction of guinea-pig isolated trachea. *Br. J. Pharmacol.*, **77**, 39–44.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative use of drugs antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BARNES, P.J., CUSS, F.M. & PALMER, J.B. (1985). The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br. J. Pharmacol.*, **86**, 685–692.

BROWN, C.M. & COLLIS, M.G. (1982). Evidence for an A_2/Ra adenosine receptor in the guinea-pig trachea. *Br. J. Pharmacol.*, **76**, 381–387.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptors. In *Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach*. ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

CHURCH, M.K., FEATHERSTONE, R.L., CUSHLEY, M.J., MANN, J.S. & HOLGATE, S.T. (1986). Relationships between adenosine, cyclic nucleotides and xanthines in asthma. *J. Allergy. Clin. Immunol.*, **78**, 670–675.

CHRISTIE, J. & SATCHELL, D.G. (1980). Purine receptors in the trachea: is there a receptor for ATP? *Br. J. Pharmacol.*, **70**, 512–514.

COLEMAN, R.A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. *Br. J. Pharmacol.*, **57**, 51–57.

COLEMAN, R.A. & LEVY, O.P. (1974). A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. *Br. J. Pharmacol.*, **52**, 167–174.

CUSHLEY, M.J., TATTERSFIELD, A.E. & HOLGATE, S.T. (1983). Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br. J. Clin. Pharmacol.*, **15**, 161–165.

CUSHLEY, M.J., TATTERSFIELD, A.E. & HOLGATE, S.T. (1984). Adenosine-induced bronchoconstriction in asthma: antagonism by inhaled theophylline. *Am. Rev. Resp. Dis.*, **129**, 380–384.

EMMERSON, J. & MACKAY, D. (1979). The zig-zag tracheal strip. *J. Pharm. Pharmacol.*, **31**, 798.

FARMER, J.B. & FARRAR, D.G. (1976). Pharmacological studies with adenine, adenosine and some phosphorylated derivative on guinea-pig tracheal muscle. *J. Pharm. Pharmacol.*, **28**, 748–752.

FARMER, S.G., FEDAN, J.S., HAY, D.W.P. & RAEBURN, D. (1986). The effect of epithelium removal on the sensitivity of guinea-pig isolated trachealis to bronchodilator drugs. *Br. J. Pharmacol.*, **89**, 407–414.

FLAVAHAN, N.A., AARHUS, L.L., RIMELE, T.J. & VANHOUTTE, P.M. (1985). Respiratory epithelium inhibits bronchial smooth muscle tone. *J. Appl. Physiol.*, **58**, 834–838.

FREDHOLM, B.B., BRODIN, K. & STRANDBERG, K. (1979). On the mechanism of relaxation of tracheal muscle by theophylline and other cyclic nucleotide phosphodiesterase inhibitors. *Acta Pharmacol. Toxicol.*, **45**, 336–344.

FROSSARD, N. & MULLER, F. (1986). Epithelial modulation of tracheal smooth muscle responses to antigenic stimulation. *J. Appl. Physiol.*, **61**, 1449–1456.

GOLDIE, R.G., PAPADIMITRIOU, J.M., PATERSON, J.W., RIGBY, P.J., SELF, H.M. & SPINA, D. (1986). Influence of the epithelium on responsiveness of guinea-pig trachea to contractile and relaxant agonists. *Br. J. Pharmacol.*, **87**, 5–14.

HAY, D.W.P., FARMER, S.G., MUCCITELLI, R.M., RAEBURN, D. & FEDAN, J.S. (1986a). Influence of the epithelium on relaxation responses of the guinea-pig trachea. *Fed. Proc.*, **45**, 985.

HAY, D.W.P., RAEBURN, D., FARMER, S.G., FLEMING, W.W. & FEDAN, J.S. (1986b). Epithelium modulates the reactivity of ovalbumin-sensitized guinea-pig airway smooth muscle. *Life Sci.*, **38**, 2461–2468.

HOLROYDE, M.C. (1986). The influence of epithelium on the responsiveness of guinea-pig isolated trachea. *Br. J. Pharmacol.*, **87**, 501–507.

KARLSSON, J.A., KJELLIN, G. & PERSSON, C.G.A. (1982). Effects on tracheal smooth muscle of adenosine and methylxanthines and their interaction. *J. Pharm. Pharmacol.*, **34**, 788–793.

KENAKIN, T.P. (1984). The classification of drugs and drug receptors in isolated tissues. *Pharmacol. Rev.*, **36,** 165–222.

KOLASSA, N., PFLEGER, K. & RUMMEL, W. (1970). Specificity of adenosine uptake into the heart and inhibition by dipyridamole. *Eur. J. Pharmacol.*, **9,** 265–268.

MANN, J.S. & HOLGATE, S.T. (1985). Specific antagonism of adenosine-induced bronchoconstriction in asthma by oral theophylline. *Br. J. Clin. Pharmacol.*, **19,** 685–692.

RAEBURN, D., HAY, D.W.P., FARMER, S.G. & FEDAN, J.S. (1986). Epithelium removal increases the reactivity of human isolated tracheal muscle to methacholine and reduces the effect of verapamil. *Eur. J. Pharmacol.*, **123,** 451–454.

TSCHIRHART, E. & LANDRY, Y. (1986). Airway epithelium does release a relaxant factor: a demonstration with substance P. *Eur. J. Pharmacol.*, **132,** 103–104.

(Received May 20, 1987.
Revised September 4, 1987.
Accepted September 25, 1987.)