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Analysis of the bronchoconstrictor responses to adenosine receptor agonists in sensitized guinea-pig lungs and trachea

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Abstract

Airway perfused lungs and half-lungs and superfused tracheal spirals from ovalbumin-sensitized guinea pigs were set up. Adenosine and the analogues, 5′-(*N*-ethylcarboxamido)adenosine (NECA), *R*-N^6-phenylisopropyladenosine (*R*-PIA), 2-chloroadenosine, N^6-2-(4-aminophenyl)ethyladenosine (APNEA) and 5′-AMP yielded bronchoconstrictor responses as increases in perfusion pressure or of tension, respectively, of these two preparations. These responses were greater in tissues from sensitized compared with un-sensitized guinea pigs. Cross-tachyphylaxis occurred between the constrictor responses to adenosine and the other constrictor adenosine agonists which indicated a common site of action. The adenosine transport inhibitors, dipyridamole and *S*-(*p*-nitrobenzyl)-6-thioinosine (NBTI), inhibited the constrictor responses to adenosine and the analogues, except 2-chloroadenosine. This was attributed to a potentiation of the opposing relaxant effects which generally occurred at higher concentrations of the agonists. The P_1 purinoceptor antagonists 8-phenyltheophylline and 8-cyclopentyltheophylline (A_1 receptor selective) failed to remove the constrictor responses to adenosine either alone or in the presence of dipyridamole. This suggests that the bronchoconstrictor response of sensitized airways tissues is mediated via the novel xanthine-resistant A_3 receptor.

Keywords: Bronchoconstriction, adenosine-induced; Tracheal spirals; (Sensitized guinea pigs); Cross-tachyphylaxis; Xanthine antagonist resistance; Transport inhibitors

1. Introduction

Adenosine usually causes bronchorelaxation when applied to isolated airways preparations (Brown and Collis, 1982; Darmani and Broadley, 1986). Tissues from animals previously sensitized to ovalbumin (Thorne and Broadley, 1992) or ragweed pollen (Ali et al., 1990a), however, display bronchoconstrictor responses to adenosine. Similar observations have been made in isolated bronchi from asthmatic subjects which show a greater sensitivity to constrictor activity by adenosine than tissues from non-asthmatics (Bjorck et al., 1992). The bronchoconstrictor responses of asthmatic human bronchi were inhibited by the A_1 selective purinoceptor antagonist 2-thio-[(1,3-dipropyl)-8-cyclopentyl]-xanthine (Bjorck et al., 1992). Similarly, the response in ragweed-sensitized rabbit trachea has been attributed to A_1 receptor stimulation (Ali et al., 1990a). In contrast, we found that the bronchoconstriction to adenosine of perfused lungs from ovalbumin-sensitized guinea pigs was not blocked by the non-selective P_1 purinoceptor antagonist, 8-phenyltheophylline (Thorne and Broadley, 1992).

A small constrictor response has also been observed by some workers in tissues from unsensitized animals. This response has been shown to be antagonized by 8-phenyltheophylline by some groups (Farmer et al., 1988) but not by others (Caparrotta et al., 1984; Ghai et al., 1987). Farmer's group claimed that the response is A_1 receptor-mediated based on a rank potency order of agonists of *R*-N^6-phenylisopropyladenosine (*R*-PIA) > N^6-cyclohexyladenosine > 2-chloroadenosine > *S*-PIA.

The present study was therefore undertaken to further examine a range of adenosine agonists in tissues from sensitized guinea pigs in an attempt to resolve these discrepancies.

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2. Methods

Male Dunkin–Hartley guinea pigs (Halls, Stafford, UK) of weight 400–500 g were fed on Special Rabbit Pellet (plain) 680 supplied by Grain Harvesters (Canterbury, UK) or Diet TR2 with vitamin C supplied by Pilsbury's (Birmingham, UK). Drinking water supplemented with ascorbic acid was allowed ad libitum.

2.1. Sensitization procedure

Guinea pigs were actively sensitized with ovalbumin administered by intraperitoneal injections on day O (5 mg) and day 2 (10 mg). They were used 14–15 days after the start of immunization.

2.2. Airway-perfused lung preparations

Guinea pigs were killed by cervical dislocation and trachea and lungs excised. The trachea was removed 5–10 mm above the bifurcation. The entire lung was perfused via the airways by cannulating the trachea. Half-lung preparations were perfused via the bronchi. Preparations were perfused with warmed (37°C) and gassed (5% CO_2 in oxygen) Krebs-bicarbonate solution of composition in twice-distilled water (mM): 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. Perfusion was at a constant flow rate (5 ml min^{-1}) maintained by a Watson–Marlow peristaltic pump (tube internal diameter 0.5 mm) and changes in back pressure were measured with a transducer (Bell and Howell, type 4-327-L221) located at the side arm on the tracheal cannula. A Condon mercury manometer was included in the system in series with the pressure transducer to accommodate some degree of volume change during drug responses. Perfusion pressure (resting level approximately 15 mm Hg) was recorded on a Devices M19 polygraph (Lectromed, Welwyn Garden City, UK). No scarification of the lung surface was necessary to allow outflow of perfusate.

2.3. Superfused tracheal spirals

The trachea was cut spirally (Constantine, 1965) and 3–4 cm lengths were suspended in a heated jacket (37°C). They were superfused with prewarmed and gassed (5% CO_2 in oxygen) Krebs-bicarbonate solution at a constant flow rate of 5 ml/min. Changes in isometric tension were measured by attaching the upper end of the spiral to a Devices UFI transducer (57 g sensitivity range) and recorded on a Devices M19 polygraph. A resting tension of 1 g was initially applied to the spirals for 45–60 min to allow the tissue to develop intrinsic tone.

2.4. Experimental protocol

After the 45–60 min equilibration period, agonists were added to lung or tracheal preparations as 0.1 ml bolus injections made into the connecting rubber tubing immediately prior to the warming coil. Dose–response curves were constructed by half-logarithmic increments in dose, each dose being added when the perfusion pressure had returned to the resting level. For construction of dose–response curves in the lungs, resting perfusion pressure was raised by perfusing with carbachol (1.0 μM) throughout. This raised the perfusion pressure by 38.5 ± 5.9 mmHg ($n = 4$) compared with the maximum constriction of 69.5 $\pm$ 6.7 mmHg obtained with dose–response curves.

Adenosine receptor antagonists or transport inhibitors were usually examined in paired lung-halves or tracheal spirals from the same animal, one half serving as the control and the other being exposed to antagonist from 30 min before and thereafter throughout agonist dosing. To eliminate differences in responses between left and right lung halves, they were alternated for antagonist exposure.

For evaluating different agonists in the same preparation, single submaximal bolus doses were usually given in the order: carbachol (10 μg lung, 30 ng trachea), adenosine (300 μg lung, 100 μg trachea) or an adenosine analogue, ovalbumin (500 ng in both). No concomitant infusion with carbachol was made during these experiments. In cross-tachyphylaxis experiments, one of the paired tissues received an adenosine agonist between the carbachol and adenosine doses.

2.5. Analysis of results

Responses were measured as the peak change in perfusion pressure (lungs) or isometric tension (trachea) induced by each bolus dose of agonist. Mean responses ($\pm$S.E.M.) were calculated. Statistical comparisons were made between responses for each dose of agonist by Student's paired or unpaired t-test, a significant difference being assumed at the 5% probability level.

2.6. Drugs

The following drugs were obtained commercially: adenosine, adenosine 5′-monophosphate (5′-AMP) (Sigma), N^6-2-(4-aminophenyl)ethyladenosine (APNEA) (Research Biochemicals), carbachol (carbamylcholine chloride), 2-chloroadenosine, 8-cyclopentyltheophylline (CPT), dipyridamole, 5′-(*N*-ethylcarboxamido)adenosine (NECA), histamine diphosphate, inosine, 5-(*p*-nitrobenzyl)-6-thioinosine (NBTI) (Sigma), ovalbumin (BDH, Dorset, UK), *R*-N^6-phenylisopropyladenosine (*R*-PIA) and 8-phenyltheophylline (8-PT) (Sigma). 2′,5′-dideoxyadenosine was a gift from Pharmacia (Buckinghamshire, UK).

Stock solutions were made up in 0.9% saline except for dipyridamole and NBTI which were initially dissolved in 0.1 ml 1 M HCl, and 8-phenyletheophylline and 8-cyclopentyltheophylline which were dissolved in 1 ml 1 M NaOH. Further dilutions of all solutions were made with saline. Bolus doses of vehicle had no effect on perfusion pressure or tension.

3. Results

3.1. Effects of adenosine and analogues in perfused lungs from untreated and ovalbumin-sensitized guinea pigs

Dose–response curves for adenosine agonists obtained in airway perfused whole lungs in which the perfusion pressure was raised by constant perfusion with carbachol are shown in Fig. 1. Relaxation responses were generally dominant, with an initial constriction at lower doses being observed with adenosine, 5′-AMP and APNEA. This component of the response was generally larger in tissues from ovalbumin-sensitized guinea pigs with adenosine and 5′-AMP.

Single submaximal doses of carbachol (10 μg), histamine (30 μg), adenosine (300 μg) and ovalbumin (500 ng) were administered, in that order, to half-lungs from untreated and ovalbumin-sensitized guinea pigs (Fig. 2). The carbachol- and histamine-induced constrictor responses did not differ between unsensitized and sensitized tissues. Adenosine, however, produced a constrictor response in the lung-halves from sensitized guinea pigs which was significantly greater than the marginal constriction seen in unsensitized tissues. Ovalbumin caused a constriction of equivalent size only in tissues from sensitized animals.

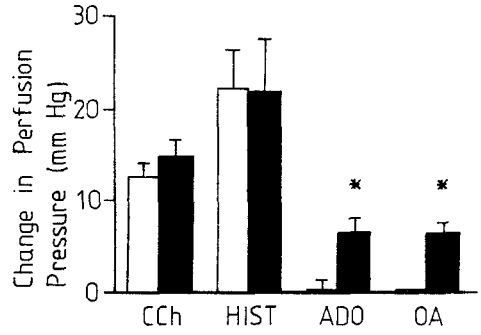

Fig. 2. Bronchoconstrictor responses to single bolus doses of carbachol (CCh, 10 μg), histamine (HIST, 30 μg), adenosine (ADO, 300 μg) and ovalbumin (OA, 500 ng) in perfused half-lungs from untreated (open histograms, $n = 6$) and ovalbumin sensitized (solid histograms $n = 6$) guinea pigs. Responses are shown as the mean (±S.E.M.) increases in perfusion pressure. * Significant difference between untreated and sensitized tissues ($P < 0.05$).

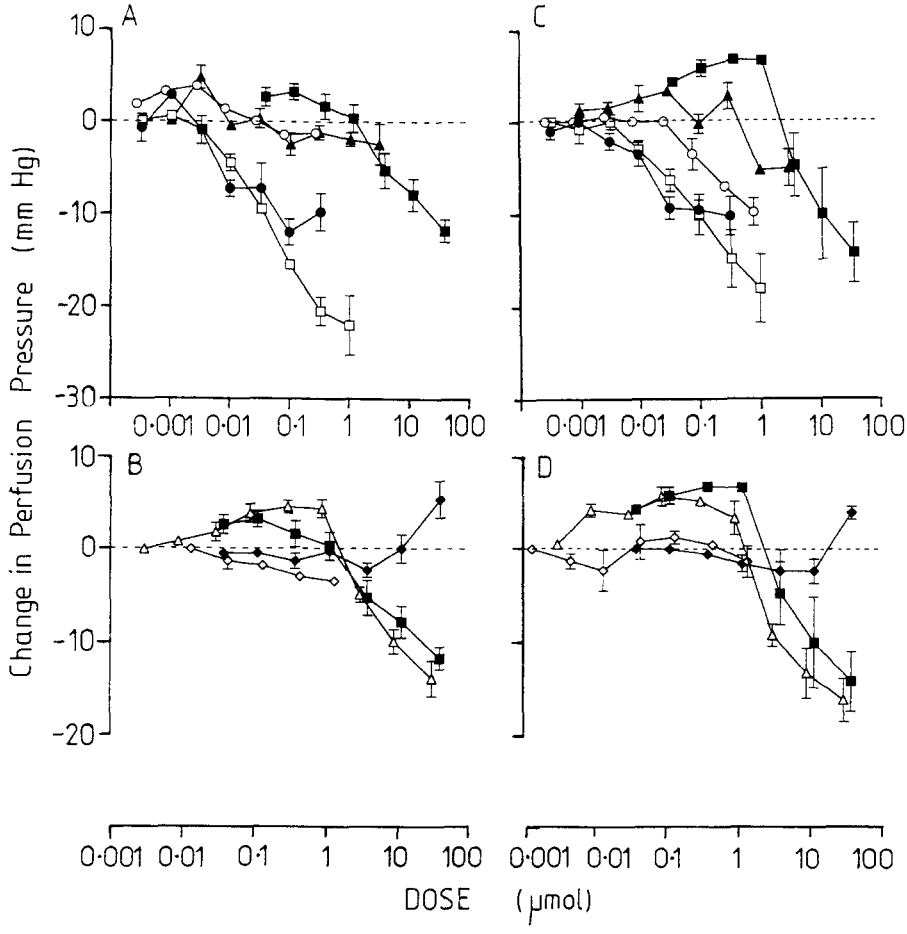

Fig. 1. Concentration–response curves for adenosine analogues in isolated perfused lungs from untreated (A and B) and ovalbumin-sensitized (C and D) guinea pigs. Each point is the mean (±S.E.M.) changes in perfusion pressure from the resting level raised by perfusing with carbachol (1 μM) ($n \geq 4$). The compounds are adenosine (■), NECA (●), *R*-PIA (○), 2-chloroadenosine (□), 5′-AMP (△), 2′,5′-dideoxyadenosine (◇), APNEA (▲) and inosine (◆).

3.2. Tachyphylaxis to the adenosine-induced constriction

Paired lung-halves from sensitized guinea pigs were set up. One half received bolus doses of carbachol (10 μg), adenosine (300 μg) and ovalbumin (500 ng), in that order, while the other half received carbachol (10 μg), an adenosine analogue, then adenosine (300 μg) and ovalbumin (500 ng). Our previous studies have shown that adenosine causes tachyphylaxis to itself (Thorne and Broadley, 1992). Comparison of the paired adenosine responses would therefore indicate whether a prior exposure to another adenosine analogue caused cross-tachyphylaxis to adenosine. The constrictor responses to adenosine showed some variation between experiments, which was probably due to seasonal and animal size differences. However, paired comparisons of the adenosine response within each group was valid. Following exposure to NECA, *R*-PIA, 2-chloroadenosine, 5′-AMP, APNEA and 2′,5′-dideoxyadenosine the response to adenosine was reduced compared with the paired control (Fig. 3), only in the latter case was this non-significant.

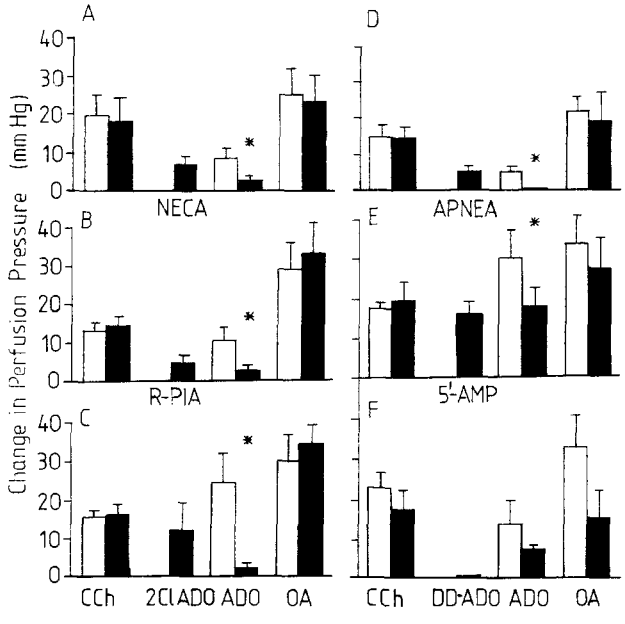

Fig. 3. Cross-tachyphylaxis between adenosine and adenosine analogues in perfused half-lungs from ovalbumin-sensitized guinea pigs. In paired half-lungs, one half (open histograms) received bolus doses of carbachol (CCh, 10 μg), adenosine (ADO, 300 μg, 0.9 μmol) and ovalbumin (OA, 500 ng) while the other half received carbachol, an adenosine analogue followed by adenosine and ovalbumin (solid histograms). The adenosine analogues were (A) NECA (300 ng, 1 nmol, $n = 5$), (B) *R*-PIA (1 μg, 2.6 nmol, $n = 6$), (C) 2-chloroadenosine (2 ClADO, 300 μg, 1 μmol, $n = 9$), (D) APNEA (300 μg, 0.8 μmol, $n = 5$), (E) 5′-AMP (300 μg, 0.9 μmol, $n = 5$) and (F) 2′,5′-dideoxyadenosine (DD-ADO, 10 μg, 42 nmol, $n = 5$). Responses are shown as the mean (±S.E.M.) increases in perfusion pressure. * Significant reduction of the adenosine responses when preceded by an adenosine analogue ($P < 0.05$).

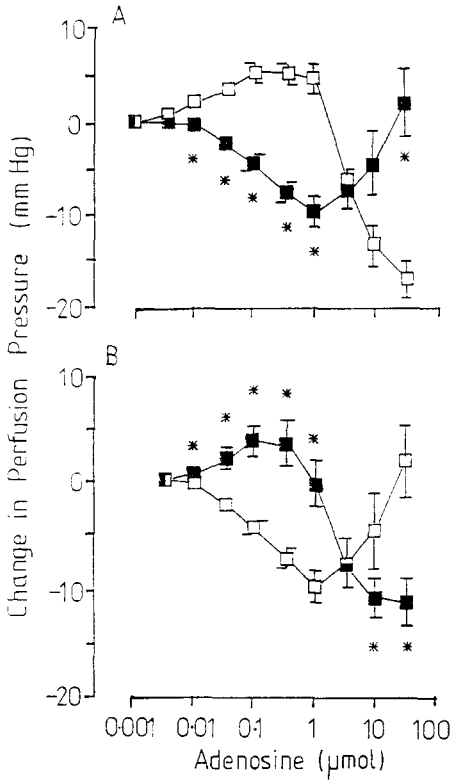

Fig. 4. Effects of (A) dipyridamole and (B) 8-phenyltheophylline in the presence of dipyridamole on the concentration-response curves for adenosine in isolated perfused half-lungs from ovalbumin-sensitized guinea pigs. Paired half-lungs were set up from each animal. In (A) one half was in the absence (open symbols) and the other half in the presence of dipyrimadole (2 μM, solid symbols, $n = 6$). In (B) both half-lungs were in the presence of dipyridamole (2 μM), one half in the absence (open symbols) and the other half in the additional presence of 8-phenyltheophylline (4 μM, solid symbol, $n = 6$). Responses are shown as the mean (±S.E.M.) change in perfusion pressure from the resting level raised by perfusing with carbachol (1 μM). * Significant difference between absence and presence of dipyridamole (A) or of 8-phenylytheophylline (B) ($P < 0.05$).

3.3. Effect of adenosine transport inhibitors on constrictor responses to adenosine analogues

Dose–response curves for adenosine were obtained in half-lungs from ovalbumin-sensitized guinea pigs perfused throughout with carbachol. In the presence of the transport inhibitor, dipyridamole (2 μM), the initial constrictor response to low doses of adenosine was no longer present (Fig. 4). Only a relaxation phase was observed (ED_{50} 0.15 (0.08–0.28) μmol) with higher doses showing a return to baseline.

Single bolus doses of adenosine were next examined, along with carbachol and ovalbumin, in paired half-lungs or tracheal spirals from sensitized guinea pigs. One half served as the control, the other was in the presence of dipyridamole (2 μM) throughout. Dipyridamole had no

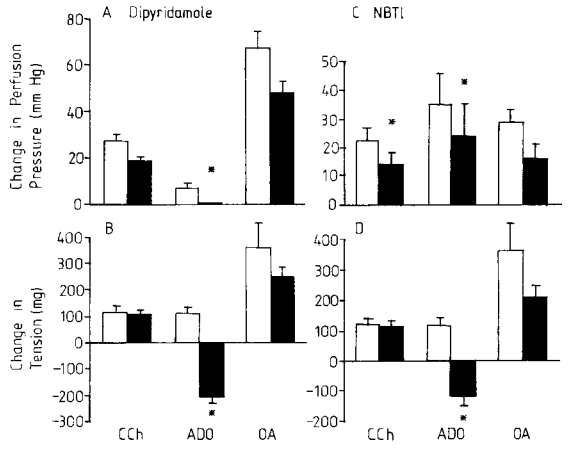

Fig. 5. Effects of dipyridamole (left panels, A and B) and 5-(p-nitrobenzyl)-6-thioinosine (NBTI) (right panels, C and D) on the responses of perfused half lungs (top panels, A and C) ($n = 5$ and 7, respectively) and superfused tracheal spirals (lower panels, B and D) ($n = 8$ and 7, respectively) from ovalbumin-sensitized guinea-pigs. Paired tissues were set up from each animal, one half was in the absence (open histograms) and the other half in the presence of dipyridamole (2 μM) or NBTI (1 μM) (solid histograms). Responses to bolus doses of carbachol (CCh, 10 μg in lungs, 30 ng in trachea), adenosine (ADO, 300 μg and 100 μg) and ovalbumin (OA, A, 200 μg and B, C and D, 500 ng) are shown as the mean ($\pm$S.E.M.) changes in perfusion pressure (lung) or tension (trachea) from the resting level. * Significant difference between absence and presence of dipyridamole or NBTI ($P < 0.05$).

significant effect on the constrictor responses to carbachol, while the ovalbumin-induced constrictions were generally reduced but not significantly. The constrictor response of the lung to adenosine was abolished (Fig. 5A). The tracheal response to adenosine was a contraction which was converted to a relaxation by dipyridamole (Fig. 5B).

Single bolus doses of NECA, *R*-PIA, 2-chloroadenosine

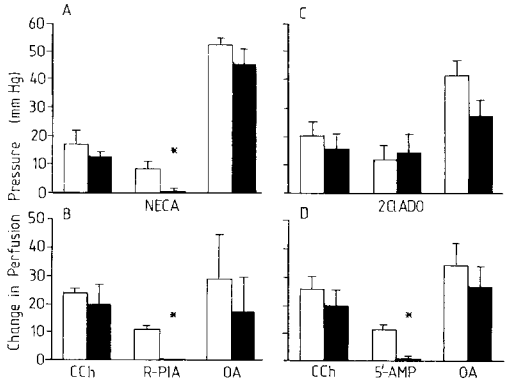

Fig. 6. Effects of dipyridamole on the responses of perfused half-lungs to bolus doses of carbachol (CCh, 10 μg), ovalbumin (OA, 500 ng) and either (A) NECA (300 ng, 1 nmol, $n = 6$), (B) *R*-PIA (1 μg, 2.6 nmol, $n = 4$), (C) 2-chloroadenosine (2 ClADO, 300 μg, 1 μmol, $n = 7$) or (D) adenosine 5'-monophosphate (5'-AMP, 300 μg, 0.9 μmol, $n = 7$). Paired half-lungs were set up from each animal, one half in the absence (open histograms) and the other half in the presence of dipyridamole (2 μM, solid histograms). Responses are shown as the mean ($\pm$S.E.M.) increases in perfusion pressure from the resting level. * Significant difference between absence and presence of dipyridamole ($P < 0.05$).

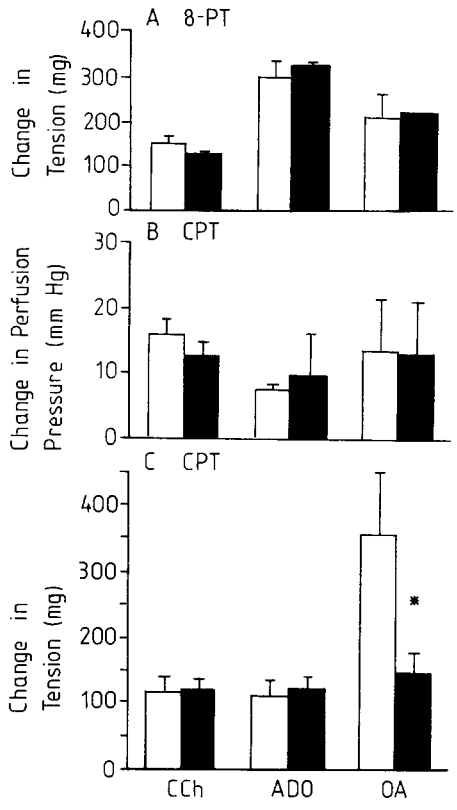

Fig. 7. Bronchoconstrictor responses of superfused tracheal spirals (A and C) and perfused half-lungs (B) from ovalbumin-sensitized guinea pigs in the absence (open histograms) and presence (solid histograms) of 8-phenyltheophylline (A, 8-PT, 4 μM, $n = 7$) or 8-cyclopentyltheophylline (B and C, CPT, 1 μM, $n = 5$ and 11). Paired tissues were set up from each animal, one half in the absence and the other half in the presence of 8-phenyltheophylline or 8-cyclopentyltheophylline. Responses to bolus doses of carbachol (CCh, 10 μg in lungs, 30 ng in trachea), adenosine (ADO, 300 μg in lungs, 100 μg in trachea) and ovalbumin (OA, 500 ng) are shown as the mean ($\pm$S.E.M.) increases in perfusion pressure (lung) or tension (trachea) from the resting level. * Significant difference between absent and presence of antagonist.

or 5'-AMP were next examined in the absence and presence of dipyridamole in perfused half-lungs. The constrictor responses to NECA, *R*-PIA and 5'-AMP were abolished by dipyridamole whereas the constriction by 2-chloroadenosine was unaffected (Fig. 6).

An alternative adenosine transport inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine (NBTI, 1 μM) was examined in half-lungs and tracheal spirals from ovalbumin sensitized guinea pigs. The constrictor response to adenosine was attenuated in the lungs by NBTI (Fig. 5C) and converted to a relaxation in the trachea (Fig. 5D). The responses to ovalbumin were reduced but not significantly and the carbachol-induced constriction in the lungs was reduced but not in the trachea.

3.4. Effects of purinoceptor antagonists on the constrictor responses to adenosine

8-phenyltheophylline (4 μM) was examined in paired tracheal spirals, one section in the absence and the other in

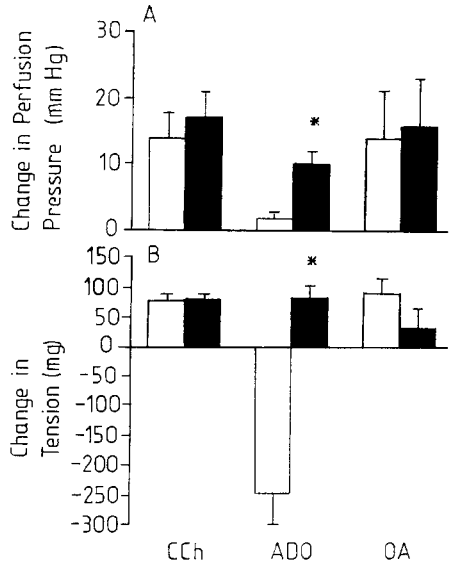

Fig. 8. Effects of 8-phenyltheophylline on the responses of (A) perfused half-lungs ($n = 7$) and (B) superfused tracheal spirals ($n = 4$) from ovalbumin-sensitized guinea pigs in the presence throughout of dipyridamole (2 μM). Paired tissues were set up from each animal both in the presence of dipyridamole, one half was in the absence (open histograms) and the other half in the additional presence of 8-phenyltheophylline (4 μM, solid histograms). Responses to bolus doses of carbachol (CCh, 10 μg in lungs, 30 ng in trachea), adenosine (ADO, 300 μg and 100 μg) and ovalbumin (OA, 500 ng in both) are shown as the mean (±S.E.M.) change from the resting level. * Significant difference between absence and presence of 8-PT ($P < 0.05$).

the presence of the antagonist. There was no effect upon the contractile responses to carbachol, adenosine or ovalbumin (Fig. 7A). This experiment was repeated in half-lungs and tracheae but with the transport inhibitor dipyridamole (2 μM) present in both tissues of the pair. Dipyridamole alone suppressed the constrictor response to adenosine in the perfused lung and converted it to a relaxation in the trachea (Fig. 8). This effect had already been observed in Fig. 5. In the combined presence of dipyridamole and 8-phenyltheophylline (4 μM), the constrictor responses to adenosine were restored. The responses to carbachol were unaffected by the additional presence of 8-phenyltheophylline.

Dose–response curves to bolus doses of adenosine were obtained in paired half-lungs, one half being perfused with dipyridamole (2 μM) alone, the other with dipyridamole and 8-phenyltheophylline (4 μM). As with the single dose study (Fig. 8), the additional presence of 8-phenyltheophylline resulted in constrictor response appearing at low doses of adenosine, followed at higher doses by a relaxation (Fig. 4B). Thus, the dose–response curve was restored to approximately the same shape as in the control half-lung (Fig. 4A). However, the relaxation phase of the curve in the combined presence of 8-phenyltheophylline and dipyridamole (ED_{50} 3.09 (2.34–4.10) μmol) was not significantly different from the control curve (ED_{50} 6.56 (3.96–10.80) μmol).

Finally, the selective A_1-adenosine receptor antagonist, 8-cyclopentyltheophylline (1 μM) was examined in paired half-lungs and tracheal spirals (Fig. 7B, C). There was no antagonism of the constrictor responses to adenosine or carbachol in either preparation by 8-cyclopentyltheophylline. The constrictor response to ovalbumin was unaffected in the lungs but significantly reduced in the trachea.

4. Discussion

Adenosine and a range of analogues caused constrictor responses of the airways perfused lungs and superfused tracheal spirals from ovalbumin-sensitized guinea pigs. This observations confirms our previous studies (Thorne and Broadley, 1992) in which we showed that sensitization of the animals reveals a constrictor response. It is also compatible with other studies showing that sensitization of rabbits to ragweed pollen causes the appearance of contractile responses of tracheal or bronchial rings (Ali et al., 1990a). These authors also showed the presence of adenosine receptor binding sites in the lungs of sensitized rabbits, but not in normal rabbits (Mustafa et al., 1992). Sensitization also reveals a bronchoconstrictor response in vivo; ovalbumin-sensitized guinea pigs (Thorne and Broadley, 1994) and ragweed-sensitized rabbits (Ali et al., 1992) display bronchoconstriction after inhaled adenosine whereas unsensitized animals show no response. This is comparable to the situation in humans where inhaled adenosine (or 5′-AMP which is converted to adenosine by the action of 5′-nucleotidase) causes bronchoconstriction of asthmatic subjects but no effect in non-asthmatic non-atopic individuals (Cushley et al., 1983; Church and Holgate, 1986). Human isolated bronchi from asthmatic subjects have been shown to be more sensitive to the contractile effects of adenosine than were bronchi from non-asthmatics (Bjorck et al., 1992).

In the present study, the dose–response curves of perfused lungs to adenosine analogues were dominated by the relaxation phase which followed the early constriction with some agonists. This was because the perfusion pressure was raised by the continuous infusion of carbachol in these experiments. The scope for further constriction by the weaker adenosine analogues was therefore limited, although the carbachol-induced constriction was submaximal (maximal increase in perfusion pressure by carbachol, 69.5 ± 6.7 mmHg). The constrictor responses were therefore relatively minor under these conditions which were therefore not appropriate for evaluating the bronchoconstriction further. However, when single doses were introduced to the perfused lung and trachea of sensitized guinea pigs without the resting pressure or tension raised with carbachol, only a constrictor response was observed. Bronchoconstriction was obtained with adenosine, NECA, *R*-PIA, APNEA, 2-chloroadenosine and 5′-AMP, the latter

because it is rapidly converted to adenosine (Pearson and Slakey, 1990). Dideoxyadenosine failed to produce a bronchoconstriction (Fig. 3). This derivative is thought to have only intracellular actions, interacting with a putative P-site (Londos and Wolff, 1977). The lack of constrictor responses suggests that the response with the other agonists is mediated via an extracellular purinoceptor.

A potency order for the contractile responses could not be determined accurately from the dose–response curves as explained above, but in unsensitized lungs the most potent agonists in this respect were *R*-PIA, NECA and APNEA, which were approximately equipotent. This would suggest an A_1 receptor mediated response (Collis and Hourani, 1993) comparable with the findings of Farmer et al. (1988). Whether the constrictor response in sensitized tissues was mediated via the same receptors is unlikely, as discussed later. The relaxation response, however, showed a potency order, NECA ≥ 2-chloroadenosine > *R*-PIA > adenosine in unsensitized lungs. This agrees with the findings of Brown and Collis (1982), Ghai et al. (1987) and Farmer et al. (1988) who classified the relaxation responses of the trachea as A_2 receptor-mediated. A recent study, however, fails to fit the tracheal relaxation response into the A_2 classification (Losinski and Alexander, 1995).

The location of the receptor mediating the constrictor response to adenosine and its analogues in sensitized tissues was further examined by use of the adenosine transport inhibitors dipyridamole and NBTI. The responses to adenosine analogues that are taken up by cells via facilitated diffusion would be expected to be potentiated by inhibitors if the response is mediated via an extracellular receptor. This is because a site of removal of the agonist from the biophase in proximity to the extracellular receptor is removed thus raising the effective concentration. Dipyridamole potentiates responses to adenosine in a wide range of tissues including the relaxation response of the trachea (Brown and Collis, 1982; Darmani and Broadley, 1986). The concentration-response curves for relaxation of the trachea by NECA, *R*-PIA and 5′-*N*-cyclopropylcarboxamide adenosine (NCPCA), however, were not potentiated. This was taken to indicate that these analogues were not substrates for the uptake pathway. The responses to 2-chloroadenosine were in fact inhibited suggesting that part of its action may be intracellular after entering the cell via the transport pathway (Brown and Collis, 1982). In the present study, the contractile responses of the lungs and trachea to adenosine and all of the analogues studied, except 2-chloroadenosine, were inhibited by dipyridamole or NBTI. In the trachea, the relaxation component of the response was revealed. Similarly, in lung halves with pressure raised by carbachol, adenosine predominantly caused bronchorelaxation (Fig. 4). These results at first sight suggest that the bronchoconstriction by these analogues is mediated via an intracellular receptor (except for 2-chloroadenosine). An alternative explanation is that the A_2-receptor-mediated-bronchorelaxation is potentiated (see Fig. 4) sufficiently to overwhelm the contraction which is therefore apparently blocked. If the potentiation was due to inhibition of the uptake process, it would only apply to agonists that are substrates for this transport system. Thus, it could not explain the effects of dipyridamole upon agonists that are not thought to be substrates, such as NECA and *R*-PIA. It is possible that these inhibitors may potentiate the relaxant effects of all analogues (except 2-chloroadenosine) by another mechanism. For example, dipyridamole can inhibit phosphodiesterase, although generally higher concentrations are required than were employed here (Lam et al., 1982). This would enhance cAMP levels arising from the A_2-receptor stimulation and potentiate the bronchorelaxation. Dipyridamole also stimulates the production of bronchorelaxant prostacyclin and inhibits synthesis of the constrictor prostanoid, thromboxane A_2 (Rivey et al., 1984). There is little information on additional properties of NBTI, but one might expect it to have some features in common with dipyridamole.

The experiments with transport inhibitors failed to provide satisfactory answers to whether the constrictor effect of adenosine is mediated via extracellular purinoceptors. To examine whether the agonists were exerting this response via a mechanism in common with adenosine, the occurrence of cross-tachyphylaxis between each agonist and adenosine was determined. We have previously shown that the bronchoconstrictor response to adenosine displays tachyphylaxis in perfused lungs and superfused tracheae (Thorne and Broadley, 1992). Others have demonstrated loss of bronchoconstrictor activity in asthmatic subjects with repeated exposure to inhaled adenosine or AMP (Phillips et al., 1989). Significant cross-tachyphylaxis occurred between adenosine and each agonist, except dideoxyadenosine. In the latter case, there was a small reduction of the adenosine response following the exposure to dideoxyadenosine. However, cross-tachyphylaxis between this agonist and adenosine would not be expected since it failed to produce a bronchoconstriction and secondly, it has an intracellular site of action compared with the other agonists. The mechanism of the tachyphylaxis has been suggested to involve desensitization of the purinoceptor by the first exposure (Phillips et al., 1989). This is unlikely, however, since the bronchorelaxant A_2 receptor-mediated responses do not display tachyphylaxis. A more feasible explanation is that the constriction is due to mediator release, probably from mast cells (reviewed by Broadley, 1995), and that these are temporarily depleted by the first exposure to the agonist. These results suggest that the agonists exert bronchoconstriction by a common mechanism.

Finally, the involvement of P_1 purinoceptors in the bronchoconstrictor response was investigated by determining its susceptibility to antagonism by 8-phenyltheophylline and 8-cyclopentyltheophylline. 8-phenyltheophylline is non-selective between A_1 and A_2 receptors whereas 8-cyclopentyltheophylline is a selective A_1 receptor antag-

onist (Bruns et al., 1986). Both antagonists failed to antagonize the constrictor response to adenosine. This confirms our earlier observation with 8-phenyltheophylline (Thorne and Broadley, 1992). Previous studies have revealed a failure to antagonize adenosine-induced responses by methylxanthines unless uptake processes are blocked by dipyridamole (Jones et al., 1980; Darmani and Broadley, 1986). Therefore, 8-phenyltheophylline was also examined in the presence of dipyridamole. As already discussed, this transport inhibitor complicated the situation by inhibiting the constrictor response in its own right and therefore the ability of 8-phenyltheophylline to antagonize the response was difficult to assess. However, in the presence of both 8-phenyltheophylline and dipyridamole, the constrictor response was restored. This was observed both in the perfused half-lungs (single doses and dose–response curves) and tracheal spirals, in the latter case a relaxation response in the presence of dipyridamole being converted to a constriction in the additional presence of 8-phenyltheophylline. Thus, the bronchoconstrictor response to adenosine was not blocked by 8-phenyltheophylline even in the presence of dipyridamole. The concentration of 8-phenyltheophylline was adequate to antagonize A_1 or A_2 purinoceptor-mediated responses since the bronchorelaxant response was clearly displaced.

The failure to antagonize the bronchoconstrictor response to adenosine by methylxanthines is a characteristic of the recently identified A_3 subclass of P_1 purinoceptor. This receptor subtype has been identified in a mast cell line where agonists including adenosine, NECA and *R*-PIA are all secretagogues causing the release of inositol phosphates and increased cytosolic levels of Ca^{2+} (Ali et al., 1990b). These agonists also markedly potentiate the secretory responses of these cells to antigen challenge (Beaven et al., 1994). The A_3 receptor of the cultured mast cell line has been further characterized by the specific binding of the A_3 receptor radioligand, [^{125}I]APNEA (Ramkumar et al., 1993). The A_3 receptor has now been cloned from sheep and rat brain cDNA sequences and shows the same characteristics of equipotency of *R*-PIA and NECA in displacing [^{125}I]APNEA binding and resistance to displacement by xanthine antagonists (Zhou et al., 1992; Linden, 1994). A_3 receptors have also been implicated in the hypotensive responses of anaesthetized rats to APNEA (Fozard and Carruthers, 1993). A suggestion that the bronchoconstrictor response is also mediated via A_3 receptors was provided by a recent study in which intravenously administered APNEA caused a bronchoconstriction in anaesthetized non-sensitized rats of the BDE strain, which was not blocked by A_1 receptor selective antagonists 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Meade, 1995) and KF 15372 and KW 3902 (Pauwels and Joos, 1995). The present results showing a failure to antagonize the bronchoconstriction to adenosine with 8-phenyltheophylline or 8-cyclopentyltheophylline and a bronchoconstriction by all agonists examined, except dideoxyadenosine, but including the A_3 receptor-selective APNEA, are consistent with this response being mediated via an A_3 receptor.

References

Ali, S., S.J. Mustafa, S.C. Bhaltia, F.L. Douglas and W.J. Metzger, 1990a, Effects of CGS-15943 on adenosine-induced bronchoconstriction in allergic rabbits, FASEB J. 4, A613.

Ali, H., J.R. Cunha-Melo, W.F. Saul and M.A. Beaven, 1990b, Activation of phospholipase C via adenosine receptors provides synergistic signals for secretion in antigen-stimulated RBL-2H3 cells. Evidence for a novel adenosine receptor, J. Biol. Chem. 265, 745.

Ali, S., S.J. Mustafa and W.J. Metzger, 1992, Adenosine-induced bronchoconstriction in an allergic rabbit model: Antagonism by theophylline aerosol, Agents Actions 37, 165.

Beaven, M.A., V., Ramkumar and H. Ali, 1994, Adenosine A_3 receptors in mast cells, Trends Pharmacol. Sci. 15, 13.

Bjorck, T., L.E. Gustafsson and S.E. Dahlen, 1992, Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine, Am. Rev. Respir. Dis. 145, 1087.

Broadley, K.J., 1995, Purines, in: Airways Smooth Muscle: Neurotransmitters, Amines, Lipid Mediators and Signal Transduction, ed. D. Raeburn and M.A. Giembycz (Birkhauser, Basel) p. 271.

Brown, C.M. and M.G. Collis, 1982, Evidence for an A_2/R_a adenosine receptor in the guinea-pig trachea, Br. J. Pharmacol. 76, 381.

Bruns, R.F., G.H. Lu and T.A. Pugsley, 1986, Characterization of the A_2 adenosine receptor labelled by [^{3}H]NECA in rat striatal membranes, Mol. Pharmacol. 29, 331.

Caparrotta, L., F. Cillo, G. Fassina and R.M. Gaion, 1984, Dual effect of (−)-N^6-phenylisopropyladenosine on guinea-pig trachea, Br. J. Pharmacol. 83, 23.

Church, M.K. and S.T. Holgate, 1986, Adenosine and asthma, Trends Pharmacol. Sci. 7, 49.

Collis, M.G. and S.M.O. Hourani, 1993, Adenosine receptor subtypes, Trends Pharmacol. Sci. 14, 360.

Constantine, J.W., 1965, The spirally cut tracheal strip preparation, J. Pharm. Pharmacol. 17, 384.

Cushley, M.J., A.E. Tattersfield and S.T. Holgate, 1983, Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects, Br. J. Clin. Pharmacol. 15, 161.

Darmani, N.A. and K.J. Broadley, 1986, Actions and interactions of adenosine, theophylline and enprofylline on the guinea-pig spirally cut trachea, Eur. J. Pharmacol. 125, 353.

Farmer, S.G., B.J. Canning and D.E. Wilkins, 1988, Adenosine receptor-mediated contraction and relaxation of guinea-pig isolated tracheal smooth muscle: Effects of adenosine antagonists, Br. J. Pharmacol. 95, 371.

Fozard, J.R. and A.M. Carruthers, 1993, Adenosine A_3 receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat, Br. J. Pharmacol. 109, 3.

Ghai, G., M.B. Zimmerman and M.F. Hopkins, 1987, Evidence for A_1 and A_2 adenosine receptors in guinea-pig trachea, Life Sci. 41, 1215.

Jones, T.R., N.M. Lefcoe and J.T. Hamilton, 1980, Pharmacological study of adenosine and related compounds on isolated guinea pig trachea: Evidence for more than one type of purine receptor, Can. J. Physiol. Pharmacol. 58, 1356.

Lam, S.C.-T., M.A. Guccione, M.A. Packham and J.F. Mustard, 1982, Effect of cAMP phosphodiesterase inhibitors on ADP-induced shape change, cAMP and nucleoside diphosphokinase activity in rabbit platelets, Thromb. Haemost. 47, 90.

Linden, J., 1994, Cloned adenosine A_3 receptors: Pharmacological properties, species differences and receptor functions, Trends Pharmacol. Sci. 15, 298.

Londos, C. and J. Wolff, 1977, Two distinct adenosine-sensitive sites on adenylate cyclase, Proc. Natl. Acad. Sci. USA 74, 5482.

Losinski, A. and S.P.H. Alexander, 1995, Adenosine receptor-mediated relaxation of guinea-pig precontracted, isolated trachea, Br. J. Pharmacol. 116, 2425.

Meade, C.J., 1995, The mechanism by which the adenosine A_3 receptor agonist APNEA induces bronchospasm, Br. J. Pharmacol. 114, 57P.

Mustafa, S.J., S. Ali and W.J. Metzger, 1992, Adenosine receptor-mediated bronchoconstriction, Int. J. Purine Pyrimidine Res. 3, 53.

Pauwels, R.A. and G.F. Joos, 1995, Characterization of the adenosine receptors in the airways, Arch. Int. Pharmacodyn. Ther. 329, 151.

Pearson, D. and L.L. Slakey, 1990, Intracellular and extracellular metabolism of adenosine and adenine nucleotides, in: Purines in Cellular Signalling: Targets for new Drugs, eds. K.A. Jacobson, J.W. Daly and V. Manganiello (Springer, New York, NY) p. 13.

Phillips, G.D., P.K. Bagga, R. Djukanovic and S.T. Holgate, 1989, The influence of refractoriness to adenosine 5′-monophosphate on allergen-provoked bronchoconstriction in asthma, Am. Rev. Respir. Dis. 140, 321.

Ramkumar, V., G.L. Stiles, M.A. Beaven and H. Ali, 1993, The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells, J. Biol. Chem. 268, 16887.

Rivey, M.P., M.R. Alexander and J.W. Taylor, 1984, Dipyridamole: A critical evaluation, Drug Intell. Clin. Pharm. 18, 869.

Thorne, J.R. and K.J. Broadley, 1992, Adenosine-induced bronchoconstriction of isolated lung and trachea from sensitized guinea-pigs, Br. J. Pharmacol. 106, 978.

Thorne, J.R. and K.J. Broadley, 1994, Adenosine-induced bronchoconstriction in conscious hyperresponsive and sensitized guinea-pigs, Am. Rev. Respir. Crit. Care Med. 149, 392.

Zhou, Q.-Y, C.Y. Li, M.E. Olah, R.A. Johnson, G.L. Stiles and O. Civelli, 1992, Molecular cloning and characterization of an adenosine receptor – The A_3 adenosine receptor, Proc. Natl. Acad. Sci. USA 89, 7432.