Analysis of the relaxant effects of AH 21–132 in guinea-pig isolated trachealis

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1 Experiments have been performed with the dual intent of analysing the mechanism by which AH 21–132 relaxes airways smooth muscle and determining whether the effects of this compound can be distinguished from those of theophylline.

2 AH 21–132 (0.25–8 μM) and theophylline (1–1000 μM) each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. The maximal effect of AH 21–132 was equivalent to that of theophylline. No evidence was obtained that the tissue became sensitized or desensitized to the action of AH 21–132.

3 Propranolol (1 μM) profoundly antagonized the tracheal relaxant action of isoprenaline but not that of AH 21–132.

4 In indomethacin (2.8 μM)-treated tissues, tone was induced by K^+ -rich (120 mM) Krebs solution, acetylcholine (ACh, 1 mM) or histamine (200 μM). Log concentration-relaxation curves for AH 21–132, isoprenaline and theophylline were all moved to the right in the presence of the spasmogens, the smallest rightward shift being induced by histamine and the greatest by ACh. While maximal effects of AH 21–132 and theophylline were unaffected by the spasmogens, that of isoprenaline was reduced by KCl and ACh.

5 In tissues treated with indomethacin (2.8 μM), AH 21–132 (0.1–100 μM) inhibited the spasmogenic effects of potassium chloride (KCl), ACh and histamine in a concentration-dependent manner. The inhibition was characterized by rightward shifts in the spasmogen concentration-effect curves with depression of their maxima.

6 In tissues treated with both indomethacin (2.8 μM) and ACh (1 mM), the removal of tracheal epithelium caused a small but significant leftward shift in the log concentration-relaxation curve for AH 21–132 but did not alter that for theophylline.

7 In tissues treated with indomethacin (2.8 μM) and maintained at 12°C, theophylline (0.1–3.2 mM) caused concentration-dependent spasm. This effect was not shared by AH 21–132.

8 AH 21–132 (0.1–1000 μM) more potently inhibited the activity of cyclic AMP-dependent than of cyclic GMP-dependent phosphodiesterase derived from homogenates of guinea-pig trachealis. Theophylline, too, inhibited these enzymes but was less potent in each case than AH 21–132 and did not exhibit selectivity for the cyclic AMP-dependent enzyme.

9 It is concluded that AH 21–132 exerts a non-specific (i.e. effective no matter what agent is used to support tone) relaxant effect on the trachealis muscle which does not involve the activation of β -adrenoceptors. The profile of the relaxant action of AH 21–132 more closely resembles that of theophylline than that of isoprenaline. However, AH 21–132 can be differentiated from theophylline in that: (a) its relaxant potency is increased by epithelial removal; (b) it does not cause tracheal spasm; (c) it exhibits selectivity as an inhibitor of cyclic AMP-dependent as opposed to cyclic GMP-dependent phosphodiesterase. It is possible that the relaxant effects of AH 21–132 are related to its ability to inhibit cyclic nucleotide phosphodiesterases.

Introduction

The pharmacological properties of ($\pm$)-*cis*-6-(*p*-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimeth-

oxy-2-methylbenzo-[c] [1,6]naphthyridine (AH 21–132) have, as yet, been the subject only of brief reports. However, this agent has been shown to relax both human and guinea-pig airways smooth muscle

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in vitro by a mechanism not involving the activation of β -adrenoceptors (Bewley & Chapman, 1988). In anaesthetized guinea-pigs AH 21-132 has also been shown to reduce the airways hyper-reactivity to bombesin which follows the intravenous infusion either of platelet activating factor (Paf) or isoprenaline (Kristersson *et al.*, 1988). Furthermore, AH 21-132 decreases Paf-induced migration of eosinophils into the lumen of guinea-pig airways (Aoki *et al.*, 1988).

Inhibition of Paf or isoprenaline-induced airways hyper-reactivity and inhibition of Paf-induced eosinophil migration may indicate that AH 21-132 should exert prophylactic activity in asthma. Accordingly it has been proposed that AH 21-132 might prove useful in the therapy of asthma both by virtue of its bronchodilator activity and of its ability to inhibit inflammatory processes in the lung which lead to the development of airways hyper-reactivity (Aoki *et al.*, 1988; Bewley & Chapman, 1988; Kristersson *et al.*, 1988).

The experiments of the present study were designed to analyse the mechanism by which AH 21-132 evokes relaxation of isolated airways smooth muscle and to determine whether the pharmacological profile of AH 21-132 acting on airways smooth muscle could be differentiated from that of methylxanthines, such as theophylline.

Methods

Guinea-pigs (350–700 g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering adipose and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis. Small segments of trachea were set up for the isometric recording of tension changes as previously described (Foster *et al.*, 1983).

Effects of relaxant agents against the spontaneous tone of the trachea

At the outset of each experiment, tissues were subjected to an imposed tension of 1 g. After 20 min, aminophylline (1 mM) was added in order to establish the recorder pen position at zero tone. The aminophylline was subsequently washed from the tissue and, when tone was fully regained, study of the relaxant drugs commenced.

Relaxant drugs were studied by the construction of cumulative concentration-effect curves. Two fold (AH 21-132) or ten fold (isoprenaline, theophylline) concentration increments were made at intervals of 4 (isoprenaline), 5 (theophylline) or 8 (AH 21-132) min. Each tissue was challenged with AH 21-132 and either isoprenaline or theophylline. When propran-

olol (1 μ M) was used as an antagonist it was added to the Krebs solution 15 min before the construction of the second concentration-effect curves of AH 21-132 and isoprenaline. Control tissues were run concurrently with test tissues but were not exposed to propranolol.

Effects of relaxants against tone induced by ACh, K⁺-rich Krebs solution or histamine

In these experiments the tissue was challenged with only one of AH 21-132, isoprenaline or theophylline. An initial log concentration-effect curve for the relaxant was constructed essentially as described above. Indomethacin (2.8 μ M) was then added to the Krebs solution to suppress spontaneous tone. Tone was subsequently induced by tissue exposure to ACh (1 mM), K⁺-rich (120 mM) Krebs solution or histamine (200 μ M). The log concentration-effect curve for the relaxant drug was then reconstructed in presence both of indomethacin and the tone-inducing agent.

Effects of AH 21-132 on the log concentration-effect curves of spasmogens

These experiments were carried out in the presence of indomethacin (2.8 μ M). In each tissue a cumulative concentration-effect curve for one of ACh, KCl or histamine was constructed. Two fold (KCl) or ten fold (ACh, histamine) concentration increments were made at intervals of 4 (ACh), 6 (histamine) or 12 (KCl) min. Following construction of the initial spasmogen concentration-effect curve, AH 21-132 (1 μ M) was added to the Krebs solution bathing test tissues, 15 min later, the spasmogen concentration-effect curve was reconstructed in the presence of AH 21-132. Subsequent curves were constructed in the presence of AH 21-132 (10, 100 and 1000 μ M). Control tissues were run concurrently but were not exposed to AH 21-132.

Effects of epithelial removal on the relaxant actions of AH 21-132 and theophylline

In these experiments a log concentration-relaxation curve was constructed either for AH 21-132, or for theophylline, in the presence of both indomethacin (2.8 μ M) and ACh (1 mM). Such curves were constructed both in tissues where the epithelium was intact and in tissues where epithelium had been removed (confirmed by histological examination) by rubbing with a wooden stick.

Assessment of the ability of theophylline and AH 21-132 to cause tracheal spasm

These experiments were carried out on tracheae treated with indomethacin (2.8 μ M) and maintained

at 12°C, essentially as described by Small *et al.* (1988). In each tissue the spasmogenic activity of AH 21-132 (0.1–1000 µM) or theophylline (0.1–3.2 mM) was assessed by constructing a sequential concentration-effect curve. Two fold (theophylline) or ten fold (AH 21-132) concentration increments were used and 15 min tissue contact was allowed for each concentration. Each tissue was finally challenged with 10 mM caffeine and spasmogenic responses were expressed in terms of the response to the caffeine standard.

Assessment of cyclic nucleotide phosphodiesterase inhibitory activity

Muscle-rich strips of trachea were prepared as previously described (Foster *et al.*, 1983). Tracheal strips from two animals were combined and homogenized with an Ildo laboratory disperser and 10 volumes of cold medium containing 100 mM Tris-HCl, 2 mM MgCl₂ and 1 mM dithiothreitol (pH 7.5). The homogenate was used within 30 min of preparation.

Cyclic AMP- and cyclic GMP-phosphodiesterase activity in the homogenate was measured by a modification of the methods of Thompson & Appleman (1971) and Rutten *et al.* (1973). The homogenate was diluted 1 in 20 and 25 µl was assayed for enzyme activity in a final volume of 100 µl containing 40 mM Tris-HCl (pH 8.0), 2.5 mM MgCl₂, 3.75 mM β-mercaptoethanol, 0.1 unit calmodulin (phosphodiesterase activator), 10 µM CaCl₂ and either 1 µM cyclic AMP with 0.2 µCi [³H]-cyclic AMP or 1 µM cyclic GMP with 0.2 µCi [³H]-cyclic GMP. In tests of enzyme inhibition the reaction mixture contained various concentrations of AH 21-132 or theophylline as required.

The reagents and homogenate were mixed on ice and the reaction was initiated by transferring the mixture to a water bath at 37°C. Following 30 min incubation the reaction was stopped by transferring the reaction vessel to a bath of boiling water for 3 min. After cooling, 20 µl of a 1 mg ml⁻¹ solution of *Ophiophagus hannah* venom was added to the reaction mixture and the mixture was incubated for 10 min. Unreacted [³H]-cyclic AMP or [³H]-cyclic GMP was removed by passing the reaction mixture through a 0.5 ml column (Bio-Rad Econocolumn) of Dowex 1 × 8 (Cl⁻) equilibrated with 1 M HCl. The column was washed with 5 ml 0.1 M NaHCO₃ and the radioactivity in the total effluent was determined by liquid scintillation counting. Less than 10% of the tritiated cyclic nucleotide was hydrolysed in any assay.

Drugs, solutions and statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The fol-

lowing substances were used: acetylcholine chloride (Sigma), AH 21-132 (Sandoz AG), aminophylline (BDH), caffeine (Sigma), histamine acid phosphate (Sigma), indomethacin (Sigma), (–)-isoprenaline hydrochloride (Sigma), potassium chloride (Hopkin & Williams), (±)-propranolol (ICI), theophylline (Sigma).

Stock solutions of ACh and indomethacin were prepared in absolute ethanol. A stock solution of (–)-isoprenaline was prepared in 0.1 M HCl and dilutions prepared from this stock contained ascorbic acid (0.57 mM) as an antioxidant. Stock solutions of all other drugs were prepared in twice-distilled water.

The Krebs solution used in all the experiments had the following composition (mM): Na⁺ 143.5, K⁺ 5.9, Ca²⁺ 2.6, Mg²⁺ 1.2, Cl⁻ 127.6, HCO₃⁻ 25, SO₄²⁻ 1.2, H₂PO₄⁻ 1.2 and glucose 11.1. The K⁺-rich Krebs solution was of identical osmolality to Krebs solution and had the following composition (mM): Na⁺ 26, K⁺ 120, Ca²⁺ 2.6, Mg²⁺ 1.2, Cl⁻ 125, HCO₃⁻ 25, SO₄²⁻ 1.2, H₂PO₄⁻ 1.2 and glucose 11.1.

The significance of differences between means was assessed by use of either a one-tailed or a two-tailed unpaired *t* test. The null hypothesis was rejected when *P* < 0.05.

Results

Effects of relaxant agents against the spontaneous tone of the trachea

AH 21-132 (0.25–8 µM) and theophylline (1–1000 µM) each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachea. AH 21-132 was the more potent relaxant agent (Table 1) but shared the same maximum effect as theophylline. Repeated log concentration-effect curves of AH 21-132 and theophylline remained constant in shape and position (Figure 1) suggesting that tachyphylaxis developed to neither agent nor did either agent sensitize the tissue to its own action.

Isoprenaline (0.001–1 µM), too, caused concentration-dependent suppression of tracheal tone. While isoprenaline was profoundly antagonized by propranolol (1 µM) the action of AH 21-132 was unaffected by blockade of β-adrenoceptors (Figure 2).

Effects of relaxant agents against tone induced by ACh, K⁺-rich Krebs solution or histamine

In the presence of indomethacin (2.8 µM) and a tone-inducing agent such as ACh (1 mM), K⁺-rich (120 mM) Krebs solution or histamine 200 µM, the log concentration-relaxation curves of AH 21-132, iso-

Table 1 The influence of various tone-inducing agents on the relaxant potency of isoprenaline, theophylline and AH 21-132 in guinea-pig isolated trachealis

	Spontaneous tone	Histamine (200 μ M)	KCl (120 mM)	ACh (1 mM)
Isoprenaline	7.69 $\pm$ 0.05	7.66 $\pm$ 0.06	6.64 $\pm$ 0.07*	6.37 $\pm$ 0.08*
Theophylline	4.15 $\pm$ 0.05	3.48 $\pm$ 0.03*	3.49 $\pm$ 0.04*	2.93 $\pm$ 0.07*
AH 21-132	5.55 $\pm$ 0.07	5.39 $\pm$ 0.09	4.64 $\pm$ 0.05*	4.09 $\pm$ 0.04*

Data are mean pD_2 values $\pm$ s.e.mean derived from at least 6 observations. * indicates a significant difference from the corresponding value under conditions of spontaneous tone. Where histamine, KCl or ACh were used to induce the tone against which the relaxant drugs were tested, the Krebs solution also contained indomethacin (2.8 μ M).

prenaline and theophylline were all moved to the right (Figures 3, 4 and 5). However, the extent of the rightward shift was dependent on the tone-inducing agent. In general the smallest rightward shift was induced by histamine and the greatest by ACh. In the case of isoprenaline and AH 21-132, histamine failed to decrease significantly the mean pD_2 value of the relaxant agent (Table 1).

While the maximal effects of AH 21-132 and theophylline were unaffected by the various tone inducing agents, the maximal relaxant effect of isoprenaline was depressed when either ACh or the K^+ -rich solution was used as the tone-inducing agent (Figure 5).

Effects of AH 21-132 on the log concentration-effect curves of spasmogens

In tissues treated with indomethacin (2.8 μ M), AH 21-132 (0.1–100 μ M) caused concentration-dependent depression of the log concentration-effect curves of KCl, ACh and histamine (Figure 6).

Effects of epithelial removal on the relaxant actions of AH 21-132 and theophylline

AH 21-132 evoked concentration-dependent relaxation of trachealis treated both with indomethacin (2.8 μ M) and with ACh (1 mM). This held true both for

Figure 1 Suppression of the spontaneous tone of guinea-pig isolated trachealis by AH 21-132 (a) and by theophylline (b). Abscissae: $-\log$ molar concentration of AH 21-132 (a) or theophylline (b). Ordinate scales: relaxation expressed as a percentage of the maximal relaxation induced by theophylline. (●) indicates the initial and (○) the second concentration-effect curve for each agonist; the two curves being constructed in the same tissues. Each data point indicates the mean value derived from at least 6 experiments, vertical bars show s.e.mean.

Figure 2 Suppression of the spontaneous tone of guinea-pig isolated trachealis by AH 21-132 (a) and by isoprenaline (b): the influence of propranolol. Abscissae: $-\log$ molar concentration of AH 21-132 (a) or isoprenaline (b). Ordinate scale: relaxation expressed as a percentage of the maximal relaxation induced by isoprenaline. (●) pooled initial log concentration-effect curve; (○) subsequent log concentration-effect curve obtained in control tissues; (▲) subsequent log concentration-effect curve obtained in test tissues treated with 1 μ M propranolol. Each data point indicates the mean of values derived from at least 6 experiments; vertical bars show s.e.mean.

Figure 3 Guinea-pig isolated trachealis: the effects of some tone-inducing agents on the relaxant action of AH 21-132. Abscissae: $-\log$ molar concentration of AH 21-132. Ordinate scales: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. The pooled initial log concentration-effect curve ($\bullet$) and the subsequent control log concentration-effect curve ($\square$) were constructed against spontaneous tone. In test tissues subsequent log concentration-effect curves were constructed in the presence of $2.8 \mu\text{M}$ indomethacin and either $200 \mu\text{M}$ histamine ($\blacktriangle$) (a), 120 mM KCl ($\blacksquare$) (b) or 1 mM ACh ($\blacklozenge$) (c). Each data point indicates the mean of values derived from at least 6 experiments; vertical bars show s.e.mean.

Figure 4 Guinea-pig isolated trachealis: the effects of some tone-inducing agents on the relaxant action of theophylline. Abscissae: $-\log$ molar concentration of theophylline. Ordinate scales: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. The pooled initial log concentration-effect curve ($\bullet$) and subsequent control log concentration-effect curve ($\square$) were constructed against spontaneous tone. In test tissues, subsequent log concentration-effect curves were constructed in the presence of $2.8 \mu\text{M}$ indomethacin and $200 \mu\text{M}$ histamine ($\blacktriangle$) (a), 120 mM KCl ($\blacksquare$) (b) or 1 mM ACh ($\blacklozenge$) (c). Each data point indicates the mean $\pm$ s.e.mean of values derived from at least 6 experiments.

tissue with intact epithelium and for tissue where the epithelium had been rubbed away. Removal of the epithelium, however, caused a small but significant leftward shift of the concentration-effect curve of AH 21-132 (Figure 7). The mean ($\pm$ s.e.mean) pD_2 values

for AH 21-132 in rubbed and intact tissues were 4.34 ± 0.05 and 4.07 ± 0.05 respectively. Theophylline, too, relaxed trachealis treated both with indomethacin ($2.8 \mu\text{M}$) and with ACh (1 mM). However, removal of the tracheal epithelium did not cause sig-

Figure 5 Guinea-pig isolated trachealis: the effects of some tone-inducing agents on the relaxant action of isoprenaline. Abscissae: $-\log$ molar concentration of isoprenaline. Ordinate scales: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. The pooled initial log concentration-effect curve ($\bullet$) and subsequent control log concentration-effect curves ($\square$) were constructed against spontaneous tone. In test tissues subsequent log concentration-effect curves were constructed in the presence of $2.8 \mu\text{M}$ indomethacin and $200 \mu\text{M}$ histamine ($\blacktriangle$) (a), 120 mM KCl ($\blacksquare$) (b) or 1 mM ACh ($\blacklozenge$) (c). Each data point indicates the mean of values derived from at least 6 experiments; vertical bars show s.e.mean.

Figure 6 Guinea-pig isolated trachealis: the effects of AH 21-132 against log concentration-spasm curves for histamine (a), KCl (b) and ACh (c). Abscissae: concentration (mm) of spasmogen (log scale). Ordinate scales: spasm evoked expressed as a percentage of the maximal spasm evoked by ACh. (●) indicates initial control log concentration-effect curve for the spasmogen; (◆), (▲) and (■) represent curves obtained after tissue equilibration with AH 21-132 0.1, 1 and 10 μM respectively. Indomethacin (2.8 μM) was present throughout. Each data point indicates the mean of values derived from at least 6 experiments. * indicates a significant ($P < 0.05$) reduction in response compared with the corresponding response in time-matched control tissues.

nificant shift in the log concentration-effect curve of theophylline (Figure 7). The pD_2 values for theophylline in rubbed and intact tissues were 2.82 ± 0.03 and 2.81 ± 0.03 respectively.

Figure 7 Guinea-pig trachealis treated with indomethacin (2.8 μM) and ACh (1 mM): the influence of epithelial removal on the relaxant actions of AH 21-132 and theophylline. Abscissae: $-\log$ molar concentration of AH 21-132 (a) or theophylline (b). Filled symbols (●) indicate tissues with intact epithelium, open symbols (○) indicate tissues denuded of epithelium. Each data point indicates the mean of values derived from at least 6 experiments. Standard error bars largely fall within the plotted symbol size and hence are not included.

Assessment of the ability of theophylline and AH 21-132 to cause tracheal spasm

In tissue treated with indomethacin (2.8 μM) and maintained at 12°C, theophylline produced concentration-dependent spasm as previously reported (Small *et al.*, 1988). The maximal effect of theophylline closely approached the (maximal) effect of the caffeine (10 mM) standard. In contrast AH 21-132 (0.1–1000 μM) was devoid of spasmogenic activity under these conditions (Figure 8).

Assessment of cyclic nucleotide phosphodiesterase inhibitory activity

Measured with 1 μM cyclic nucleotide as substrate in the presence of Ca^{2+} and calmodulin, the muscle-rich strips of trachea contained phosphodiesterase activity equivalent to the hydrolysis of 1.8 ± 0.2 nmol cyclic AMP $\text{g}^{-1} \text{min}^{-1}$ or 2.8 ± 0.2 nmol cyclic GMP $\text{g}^{-1} \text{min}^{-1}$.

AH 21-132 and theophylline each caused concentration-dependent inhibition of the hydrolysis of cyclic AMP and cyclic GMP. AH 21-132 was the more potent PDE inhibitor in each case and, in contrast to theophylline, exhibited selectivity as an inhibitor of cyclic AMP-PDE as opposed to cyclic GMP-PDE (Table 2 and Figure 9).

Figure 8 The spasmogenic activity of theophylline and AH 21-132 in guinea-pig isolated trachealis treated with indomethacin ($2.8\mu\text{M}$) and maintained at 12°C . Abscissa scale: molar concentration of theophylline (■) or AH 21-132 (●) (log scale). Ordinate scale: spasm expressed as a percentage of the spasm evoked by caffeine (10mM). Each data point indicates the mean of values from at least 6 experiments; vertical bars show s.e.mean.

Discussion

The tracheal relaxant action of AH 21-132

We have observed that AH 21-132 suppresses the spontaneous tone of the trachea and suppresses tone induced by histamine, KCl or ACh. The relaxant action of AH 21-132 is not antagonized by propranolol. We conclude that AH 21-132 exerts a non-specific relaxant effect on the trachealis which is not mediated by the activation of β -adrenoceptors. Our findings in this respect both confirm and extend the recent report of Bewley & Chapman (1988).

Since AH 21-132 suppressed the spontaneous tone of the trachea and similarly depressed the log concentration-spasm curves for KCl, histamine and ACh, it is unlikely that AH 21-132 acts as an inhibitor of Ca^{2+} influx through voltage-operated channels. Agents that do this include nifedipine and

Figure 9 Inhibition by AH 21-132 (●) and by theophylline (■) of cyclic AMP-dependent (a) and cyclic GMP-dependent (b) phosphodiesterase of muscle rich strips of guinea-pig trachea. Abscissae: $-\log$ molar concentration of inhibitor. Ordinate scale: enzyme activity (cyclic AMP-PDE in a; cyclic GMP-PDE in b) expressed as a percentage of control activity. Each data point indicates the mean of values from 6 experiments; vertical bars indicate s.e.mean.

verapamil. They have relatively little effect against the spontaneous tone of the trachea and depress the log concentration-spasm curve for KCl at concentrations that do not modify the corresponding curves for histamine and ACh (Foster *et al.*, 1984; Ahmed *et al.*, 1985; Small & Foster, 1986).

The K^+ -channel opener, cromakalim, suppresses the spontaneous tone of guinea-pig trachea but is ineffective against tone induced by K^+ -rich (120mM) Krebs solution (Allen *et al.*, 1986a). Since AH 21-132 retained its relaxant activity in K^+ -rich (120mM) Krebs solution, it is unlikely that AH 21-132 shares the same mechanism of action as cromakalim. The present experiments showed, instead, that the relaxant action of AH 21-132 resembled that of theophylline. Nevertheless, the effects of AH 21-132 in the trachea can be distinguished from those of theophylline (see below).

Phosphodiesterase inhibition by AH 21-132: its possible role in the relaxant action

Tested on the soluble PDE of rat brain and with cyclic AMP as a substrate, AH 21-132 has been

Table 2 Inhibition of phosphodiesterase activity of homogenates of muscle-rich strips of guinea-pig trachea by AH 21-132 and theophylline

	Cyclic AMP-PDE	Cyclic GMP-PDE
AH 21-132	5.4 ± 0.20	$4.16 \pm 0.11^*$
Theophylline	3.44 ± 0.09	3.35 ± 0.24

Data are mean ($\pm$ s.e.mean) values of $-\log$ molar IC_{50} derived from 6 tissue samples. * indicates a significant ($P < 0.001$) difference between mean $-\log$ molar IC_{50} values for cyclic AMP-PDE and cyclic GMP-PDE.

reported to act as a competitive PDE inhibitor with a K_i value of $6\text{ }\mu\text{M}$ (Markstein *et al.*, 1984). The present experiments have confirmed that AH 21-132 has PDE inhibitory activity and suggest that AH 21-132 exerts some selectivity as an inhibitor of cyclic AMP-PDE as opposed to cyclic GMP-PDE unlike theophylline which appeared to be approximately equipotent in this respect.

Our enzyme inhibition studies showed that AH 21-132 is effective in inhibiting PDE over the concentration range $0.1\text{--}1000\text{ }\mu\text{M}$. Since this encompasses the effective concentration range of AH 21-132 as a tracheal relaxant, it is possible that PDE inhibition plays an important role in the process by which relaxation is achieved.

Airways smooth muscle contains PDE which exists as several different isozymes. Amongst these the type Ic isozyme (which is Ca^{2+} - and calmodulin-dependent) accounts for the majority (85%) of the total cyclic AMP hydrolytic activity of tissue homogenates. However, this isozyme may not be important for regulating the cyclic AMP content and mechanical activity of intact tissue. In contrast the type III and type IV isozymes, while accounting for only 5% and 10% respectively of the total cyclic AMP hydrolytic activity of tissue homogenates, may nevertheless be important regulators of cyclic AMP content and mechanical activity in intact tissue (Torphy, 1988). The present enzyme inhibition studies suggest that AH 21-132 can inhibit the type Ic isozyme but whether this agent inhibits (with greater or lesser potency) the type III and type IV isozymes remains to be determined.

Differences between the pharmacological profiles of AH 21-132 and theophylline acting on the trachealis

Results of the present study have confirmed an earlier report (Bewley & Chapman, 1988) that epithelial removal potentiates AH 21-132 in relaxing trachealis muscle but does not modify the actions of methylxanthines such as aminophylline or theophylline.

There are numerous reports (reviewed by Goldie *et al.*, 1988) that the airways epithelium releases a relaxant substance (epithelium-derived relaxant factor, EpDRF) which can modulate the activity of the airways smooth muscle. It is difficult to envisage a mechanism by which AH 21-132 could interfere with the release or action of EpDRF and where epi-

thelial removal would consequently potentiate AH 21-132. It is perhaps more likely that the airways epithelium represents a site of loss of AH 21-132 (by binding or by metabolism) or presents a barrier to the diffusion of the drug into the tracheal tissue. Removal of a site of loss or a diffusion barrier could certainly explain the potentiation of AH 21-132 which occurs on epithelial removal. Presumably, the epithelium represents no site of loss or barrier to diffusion for the methylxanthines. In any event, the effects of epithelial removal serve to discriminate between the tracheal relaxant actions of AH 21-132 and the methylxanthines.

The discrimination between AH 21-132 and methylxanthines provided by the differing effects of epithelial removal is reinforced by the present experiments where AH 21-132 and theophylline were tested in trachea treated with indomethacin ($2.8\text{ }\mu\text{M}$) and maintained at 12°C . Theophylline was spasmogenic but AH 21-132 was devoid of spasmogenic activity.

In trachea treated with indomethacin and maintained at 12°C , xanthine and some of its alkylated derivatives evoke spasm by releasing Ca^{2+} from intracellular sites of sequestration. This action does not depend on the intracellular accumulation of cyclic nucleotides (Small *et al.*, 1988). The present failure of AH 21-132 to mimic the spasmogenic effects of theophylline is consistent with the fact that the AH 21-132 molecule does not contain a xanthine nucleus.

In summary, while the tracheal relaxant action of AH 21-132 in many ways resembles that of the methylxanthines, the actions of AH 21-132 on the trachea could be distinguished from those of methylxanthines in three ways. Firstly, epithelial removal potentiated AH 21-132 in relaxing the trachea but did not potentiate methylxanthines. Secondly, AH 21-132 failed to mimic the tracheal spasmogenic effects of the methylxanthines. Thirdly, AH 21-132 exhibited selectivity as an inhibitor of tracheal cyclic AMP-PDE while theophylline inhibits cyclic AMP-PDE and cyclic GMP-PDE with equal potency.

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