Inhibitory effects of AH 21–132 in guinea-pig isolated ileum and taenia caeci

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1 AH 21–132 is being investigated as a potential chemotherapeutic agent for bronchial asthma. The present experiments were designed to determine whether AH 21–132 shares the activity of theophylline as an antagonist at adenosine A₁ receptors and to assess its potency as a relaxant in intestinal smooth muscle.

2 In the transmurally-stimulated guinea-pig ileum, theophylline (1 mM), but not AH 21–132 (1 and 10 μ M), antagonized twitch depression induced by adenosine. Higher concentrations (100 μ M and 1 mM) of AH 21–132 themselves had a depressant effect. Neither theophylline (1 mM) nor AH 21–132 (1 and 10 μ M) antagonized twitch depression induced by noradrenaline.

3 AH 21–132 (100 μ M and 1 mM) depressed maximum contractions of ileum induced by both acetylcholine (ACh) and histamine.

4 In ileum treated with hyoscine (1 μ M), AH 21–132 (> 10 μ M) caused a concentration-dependent depression of the log concentration-effect curve for potassium chloride.

5 Simultaneous extracellular electrophysiological and mechanical recording from taenia caeci showed that AH 21–132 (100 μ M–1 mM) inhibited spontaneous tension waves and their associated bursts of electrical spike activity.

6 Intracellular electrophysiological recording from taenia caeci showed that the mechano-inhibitory effect of 1 mM AH 21–132 was accompanied by abolition of spontaneous spike activity. Following spike abolition, the membrane potential assumed a value very close to that observed during periods of electrical quiescence prior to drug exposure.

7 AH 21–132 inhibited the activity of cyclic AMP-dependent and cyclic GMP-dependent phosphodiesterases derived from homogenates of ileal smooth muscle. The effective concentration ranges were 0.1–1000 μ M and 1–1000 μ M, respectively. Theophylline, too, inhibited these enzymes but in each case was less potent than AH 21–132.

8 It is concluded that AH 21–132 is devoid of antagonist activity at adenosine A₁ receptors which modulate ACh release from intramural cholinergic nerves in the ileum. At concentrations greater than 10 μ M, AH 21–132 has a relaxant effect on intestinal smooth muscle characterized by suppression of spontaneous action potentials but by minor change in resting membrane potential. AH 21–132 previously has been reported to depress the spontaneous tone of trachealis muscle with an EC₅₀ value of less than 10 μ M and the present experiments therefore show that this agent is much less potent in inhibiting intestinal muscle. This potency difference cannot be attributed to a tissue-related difference in the potency of AH 21–132 as an inhibitor of cyclic AMP- or cyclic GMP-dependent phosphodiesterases.

Introduction

($\pm$)-*cis*-6-(*p*-Acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo-[C][1,6]naphthyridine (AH 21–132) exerts a non-specific relaxant effect in airways smooth muscle *in vitro* (Bewley &

Chapman, 1988; Small *et al.*, 1989). In many ways the tracheal relaxant action of AH 21–132 resembles that of methylxanthines but, whereas removal of the tracheal epithelium increases the relaxant potency of AH 21–132, it does not alter the relaxant potency of aminophylline or theophylline (Bewley & Chapman,

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1988; Small *et al.*, 1989). AH 21-132 can also be distinguished from theophylline in that it does not share the latter's ability to cause tracheal spasm (Small *et al.*, 1988) by releasing Ca^{2+} from intracellular sites of sequestration (Small *et al.*, 1989).

The present experiments were performed in order to determine (a) whether AH 21-132 shares the activity of theophylline as an antagonist at adenosine A_1 receptors and (b) whether the non-specific relaxant effects of AH 21-132 observed in airways smooth muscle could also be found in intestinal smooth muscle. Our findings suggest that AH 21-132 is not an antagonist at adenosine A_1 receptors and that its potency as a relaxant of gastrointestinal smooth muscle is less than its potency as a relaxant of airways smooth muscle.

Methods

Guinea-pigs (350–700 g) of either sex were killed by stunning and bleeding. Segments of ileum and taenia caeci were removed from the animals and incubated in Krebs solution gassed with 95% O_2 and 5% CO_2 and maintained at 37°C.

Experiments with ileum

Segments of ileum 1–2 cm long were set up for isometric recording of mechanical activity under a resting tension of 1 g. The effects of acetylcholine (ACh) and histamine were studied by the construction of sequential concentration-effect curves. For each concentration of these drugs, the contact time was 45 s in a 4 min cycle; four fold increments in agonist concentration were used. Each tissue was challenged with only one agonist.

Following the construction of the initial agonist concentration-effect curve, test tissues were pretreated for 15 min with AH 21-132 before the construction of a subsequent concentration-effect curve for the agonist in its presence. The concentration of AH 21-132 was then increased before the next concentration-effect curve for the contractile agonist was constructed. Control tissues were not exposed to AH 21-132 and were run concurrently with test preparations.

A further group of experiments was performed on ileum treated with 1 μM hyoscine. KCl (5–80 mM; see below) was used as the agonist, each concentration being allowed 20 s tissue contact. The effects of AH 21-132 (1 μM –1 mM) on the KCl log concentration-effect curve were investigated in a manner similar to that described for the experiments with ACh and histamine.

Experiments with transmurally stimulated guinea-pig ileum

Segments of ileum, 1 to 2 cm long, were set up for transmural stimulation (Paton, 1955) with a resting tension of 1 g and isometric contractions of the longitudinal muscle were recorded. Transmural, supramaximal stimulation was carried out using constant voltage (60 V) pulses of 0.5 ms duration, at a frequency of 0.2 Hz. The effects of adenosine and noradrenaline on the twitches evoked by transmural stimulation were studied by the construction of cumulative concentration-effect curves, ten fold increments in agonist concentration being made at 45 s intervals. In test tissues, agonist action was studied both in the absence and presence (30 min preincubation) of AH 21-132 or theophylline. Control tissues were exposed to neither AH 21-132 nor theophylline and were run concurrently with test preparations.

Extracellular electrophysiological recording from the taenia caeci

Segments of taenia caeci 1–2 cm in length were set up for the simultaneous extracellular recording of electrical activity and tension changes by the perfused capillary method of Golenhofen & V. Loh (1970). Electrical and mechanical changes were displayed on a Grass polygraph and the time constant of the a.c. amplifier used to record the electrical activity was set at 100 ms. This enabled the recording of electrical spike activity in the absence of slower changes in membrane potential. Mechanical activity was recorded isometrically with a resting tension of 1 g.

Following a 45 min preincubation period, the effects of AH 21-132 on spontaneous electrical and mechanical activity were examined by the construction of a cumulative log concentration-effect curve. AH 21-132 was added to the Krebs solution superfusing the tissue. The initial concentration of AH 21-132 studied was 1 μM and the concentration of AH 21-132 was raised in ten fold steps with a tissue contact time of 15 min for each concentration step. The maximal concentration of AH 21-132 applied to the tissue was 1 mM. Thereafter the tissue was washed with drug-free Krebs solution so that recovery from the effect of AH 21-132 could be observed.

Intracellular electrophysiological recording from the taenia caeci

Simultaneous intracellular recordings of membrane potential changes and mechanical activity were made by the method of Small & Weston (1977; 1980). Segments of tissue approximately 2.5 cm in length were used. Mechanical activity was recorded isometrically

at an initial resting tension of 1 g. Following the microelectrode impalement of a smooth muscle cell, several minutes were allowed to elapse both to check the stability of the electrical recording and to monitor activity under control conditions. AH 21-132 (1 mM) was then added to the Krebs solution superfusing the tissue. This concentration of AH 21-132 was chosen on the basis that, in the extracellular electrophysiological studies, it clearly abolished spontaneous spike activity.

The effects of AH 21-132 on electrical and mechanical activity were monitored, where possible, over a 15 min period. For many recordings the electrode became dislodged from the cell before the 15 min drug equilibration period was complete.

Assessment of cyclic nucleotide phosphodiesterase inhibitory activity

Strips of smooth muscle were prepared from guinea-pig ileum by a method similar to that described for rabbit small intestine (Ambache, 1954). Histological examination revealed that these tissue strips contained serosa, longitudinal smooth muscle and elements of the myenteric plexus; some circular smooth muscle but no mucosal tissue was included in the tissue strips. The tissue strips were processed and the effects of AH 21-132 and theophylline on cyclic nucleotide phosphodiesterase activity were assessed as previously described (Small *et al.*, 1989).

Drugs, solutions and statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The following substances were used: acetylcholine chloride (Sigma), adenosine (Sigma), AH 21-132 (Sandoz, AG), ascorbic acid (Sigma), histamine acid phosphate (Sigma), hyoscine hydrobromide (Sigma), (–)-noradrenaline bitartrate (Sigma), potassium chloride (Analar: Hopkin and Williams), theophylline (Sigma). Where KCl was used as a contractile agonist, the stated bath concentration indicates the concentration of KCl in excess of the concentration of K^+ and Cl^- provided by the Krebs solution.

Dilutions of noradrenaline were prepared from a stock solution in 0.1 N HCl on the day of use. Each noradrenaline dilution contained ascorbic acid (0.57 mM) as an antioxidant. Dilutions of adenosine were prepared by suspending or dissolving the solid in a 0.9% (w/v) solution of NaCl immediately before use. Stock solutions of ACh were prepared in absolute ethanol, those of other drugs in twice-distilled water.

The Krebs solution used in all the experiments had the following composition (mM): Na^+ 143.5,

K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 127.6, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1.

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

Results

Experiments with ileum

ACh (0.004–4 μ M) and histamine (0.004–4 μ M) each caused concentration-dependent contraction of the longitudinal muscle of the isolated ileum. Control experiments revealed no time-dependent changes in the shape or position of the log concentration-effect curves for either ACh or histamine.

AH 21-132 (0.1–10 μ M) caused no changes in ileal tone and affected neither the slope nor the position of the log concentration-effect curves of ACh or histamine. In contrast, at high concentrations (100 μ M) AH 21-132 depressed the log concentration-effect curves for both ACh and histamine, significantly reducing maximum responses to each of these agonists (Figure 1).

In preparations treated with hyoscine (1 μ M), KCl (5–80 mM) caused concentration-dependent contraction. AH 21-132 (1–1000 μ M) caused concentration-

Figure 1 Effects of AH 21-132 on contractions induced by acetylcholine (ACh) (a) and histamine (b) in guinea-pig isolated ileum. Abscissae: concentrations (μ M) of ACh (a) or histamine (b) on a log scale. Ordinates: contraction expressed as a percentage of control maximum. Concentration-effect curves are shown prior to tissue treatment with AH 21-132 ($\circ$) and after 15 min incubation with, and in the presence of 0.1 ($\bullet$), 1 (Δ), 10 ($\blacktriangle$) and 100 ($\blacksquare$) μ M AH 21-132. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical bars. (*) indicates a significant ($P < 0.05$) reduction in response compared with the corresponding point for control tissues.

Figure 2 Effects of AH 21-132 on contractions induced by KCl in guinea-pig isolated ileum. Hyoscine ($1 \mu\text{M}$) was present throughout. Abscissa scale: concentration (mM) of KCl on a log scale. Ordinate scale: contraction expressed as a percentage of maximum. Concentration-effect curves are shown prior to tissue treatment with AH 21-132 ($\circ$) and after 15 min incubation with, and in the presence of 1 ($\bullet$), 10 (Δ), 100 ($\blacktriangle$) and 1000 ($\blacksquare$) μM AH 21-132. Data represent the means of values from at least 6 tissues; s.e.mean shown by vertical bars. (*) indicates a significant ($P < 0.05$) reduction in response compared with the corresponding point for control tissues.

dependent depression of the log concentration-effect curve for KCl, significant depression of the curve occurring at AH 21-132 concentrations of 100 and $1000 \mu\text{M}$ (Figure 2).

Experiments with transmurally-stimulated guinea-pig ileum

Noradrenaline (1 nM – $1 \mu\text{M}$) and adenosine (10 nM – $10 \mu\text{M}$) each produced concentration-dependent suppression of ileal twitches evoked by transmural stimulation (Figure 3). Theophylline (1 mM) itself had no significant effect on twitch amplitude and did not reduce the ability of noradrenaline to evoke twitch suppression. However, in the presence of theophylline, the inhibitory log concentration-effect curve for adenosine was moved (approximately ten fold) to the right (Figure 3). Concurrent control experiments revealed little or no changes in the slopes or positions of the log concentration-effect curves of noradrenaline or adenosine. Hence the reduced potency of adenosine (but not of noradrenaline) observed in

Figure 3 Inhibition of twitches of transmurally stimulated guinea-pig ileum produced by noradrenaline (left-hand panels) or adenosine (right-hand panels): ($\circ$) pooled, initial log concentration-inhibition curves for control and test tissues; ($\bullet$) log concentration-inhibition curves obtained in control tissues after treatment with vehicle; ($\blacksquare$) log concentration-inhibition curves obtained in test tissues treated with 1 mM theophylline (row a) or with $10 \mu\text{M}$ AH 21-132 (row b). Each data point is the mean of at least six experiments; vertical bars show s.e.mean.

The columns show twitch height expressed as a % of the twitch height observed prior to tissue exposure to vehicle (control tissues; open columns) or to 1 mM theophylline (stippled columns; row a) or to $10 \mu\text{M}$ AH 21-132 (stippled columns; row b).

* Indicates a significant ($P < 0.05$) difference from the corresponding data point for the time-matched control tissues.

the presence of theophylline was indicative of selective antagonism by theophylline.

AH 21-132 (1 and $10 \mu\text{M}$) itself had no significant effect on twitch amplitude and did not reduce the ability of either noradrenaline or adenosine to cause twitch suppression. Indeed, in the presence of $10 \mu\text{M}$ AH 21-132, the inhibitory log concentration-effect curves of both noradrenaline and adenosine were moved (approximately four fold and two fold, respectively) to the left (Figure 3). Higher concentrations ($100 \mu\text{M}$ and 1 mM) of AH 21-132 themselves caused suppression of ileal twitches.

Figure 4 Effects of AH 21-132 on the extracellularly-recorded electrical (upper trace) and mechanical (lower trace) activity of guinea-pig taenia caeci. Recordings from two separate (a and b) preparations are shown. Panel (i) shows control activity. Panels (ii) and (iii) show activity recorded 12 min after the cumulative administration of AH 21-132 0.1 and 1 mM respectively. Panel (iv) shows activity recorded 20 min after drug washout.

Extracellular electrophysiological recording from taenia caeci

The spontaneous activity of the taenia caeci comprised phasic tension waves and associated bursts of electrical spike activity. The frequency and duration of the tension waves and spike bursts were somewhat variable (Figure 4a and b).

AH 21-132 (1–10 μ M) had little or no effect on the ongoing electrical and mechanical activity of the taenia caeci. AH 21-132 (100 μ M), in some preparations, reduced the magnitude and/or duration of the tension waves and spike bursts (e.g. Figure 4a). In all preparations, AH 21-132 (1 mM) abolished both the spontaneous tension waves and their associated spike bursts (Figure 4a and b).

Intracellular electrophysiological recording from the taenia caeci

In the extracellular electrophysiological experiments, AH 21-132 consistently abolished spontaneous spike activity only at a concentration of 1 mM. Accordingly, this concentration of AH 21-132 was chosen for use in the intracellular electrophysiological experiments.

In all tissues examined, AH 21-132 (1 mM) abolished intracellularly-recorded spike potentials and phasic tension waves. Spike abolition generally became manifest 2–3 min after tissue exposure to AH 21-132 (Figure 5). During AH 21-132-induced spike suppression the membrane potential assumed a

Figure 5 Effects of AH 21-132 on the intracellularly-recorded electrical (upper trace) and mechanical (lower trace) activity of guinea-pig taenia caeci. Recordings from two separate (a and b) preparations are shown. Panel (i) shows control activity. Panels (ii), (iii) and (iv) show activity recorded 1, 2 and 8 min, respectively, after the addition of 1 mM AH 21-132. In each row the electrical traces are taken from a continuous recording from a single cell.

level close to, or marginally more negative than, the level of membrane potential measured between successive spike bursts during the pre-drug control period (Table 1). In other words, AH 21-132 caused

Table 1 Effects of AH 21-132 (1 mM) on resting membrane potential of guinea-pig taenia caeci

Time after administration of AH 21-132 (min)	Increase in membrane potential over the level assumed between spike bursts during the pre-drug control period (mV)
1	0.11 $\pm$ 0.6 (9)
2	1.22 $\pm$ 0.6 (9)
4	0.38 $\pm$ 1.7 (8)
8	0.00 $\pm$ 1.2 (4)

Data are mean ($\pm$ s.e. mean) increases in membrane potential. A single recording was obtained from each tissue which was subjected to a single exposure to AH 21-132. The figures in parentheses indicate the number of tissues contributing to the mean. The decrease in group size with time reflects the duration of individual recordings.

Figure 6 Inhibition by AH 21-132 (●) and by theophylline (■) of cyclic AMP-dependent (a) and cyclic GMP-dependent (b) phosphodiesterase of smooth muscle from guinea-pig ileum. The abscissa scale indicates $-\log$ molar concentration of inhibitor. The ordinate scale indicates enzyme activity expressed as a percentage of control activity. Data points represent means of values from 6 experiments; vertical bars indicate s.e.mean.

very little, if any, hyperpolarization of the smooth muscle cells of the taenia caeci.

Inhibition of ileal cyclic nucleotide phosphodiesterase by AH 21-132 and theophylline

Measured with $1 \mu\text{M}$ cyclic nucleotide as substrate, in the presence of Ca^{2+} and calmodulin, the strips of ileal smooth muscle contained phosphodiesterase activity equivalent to the hydrolysis of 5.66 ± 0.87 ($n = 6$) nmol cyclic AMP $\text{g}^{-1} \text{min}^{-1}$ or 5.15 ± 0.34 ($n = 6$) nmol cyclic GMP $\text{g}^{-1} \text{min}^{-1}$.

AH 21-132 ($1\text{--}1000 \mu\text{M}$) and theophylline ($0.4\text{--}4000 \mu\text{M}$) each caused concentration-dependent inhibition of the hydrolysis of cyclic AMP. AH 21-132 was the more potent inhibitor of cyclic AMP-dependent phosphodiesterase (Figure 6 and Table 2). AH 21-132 ($0.1\text{--}1000 \mu\text{M}$) and theophylline (0.4--

$4000 \mu\text{M}$) each caused concentration-dependent inhibition of the hydrolysis of cyclic GMP. AH 21-132 was, again, the more potent inhibitor of cyclic GMP-dependent phosphodiesterase (Figure 6 and Table 2). Furthermore, in contrast to theophylline, AH 21-132 was significantly more potent in inhibiting cyclic AMP-dependent PDE than cyclic GMP-dependent PDE.

Discussion

Effects of AH 21-132 on intestinal smooth muscle

The present experiments with guinea-pig ileum showed that, in concentrations of $100 \mu\text{M}$ or greater, AH 21-132 was able to depress log concentration-effect curves for ACh, histamine and KCl. Since the experiments with KCl were carried out in the presence of hyoscine ($1 \mu\text{M}$), we ascribe the spasm induced by KCl to a direct effect of KCl in depolarizing the smooth muscle cells rather than to the release of ACh from intramural nerves. Accordingly, the ability of AH 21-132 to suppress concentration-effect curves for ACh, histamine and KCl suggests that AH 21-132 exerts a non-specific relaxant effect on ileal smooth muscle. Such an effect would explain why, in the experiments with transmurally-stimulated ileum, ileal twitches were suppressed when the concentration of AH 21-132 was raised from 10 to $100 \mu\text{M}$. Non-specific relaxation of intestinal smooth muscle would also explain the ability of AH 21-132 ($100 \mu\text{M}\text{--}1 \text{ mM}$) to suppress the spontaneous tension waves and electrical spike activity of the taenia caeci.

In airways smooth muscle AH 21-132 exerts non-specific relaxant effects in the concentration-range $0.1\text{--}10 \mu\text{M}$ (Bewley & Chapman, 1988; Small *et al.*, 1989). In the present study AH 21-132 exerted relaxant effects on intestinal smooth muscle only in concentration greater than $10 \mu\text{M}$. This suggests that AH 21-132 exhibits some selectivity for smooth muscle of the airways as opposed to that of the intestine.

Table 2 Inhibition of phosphodiesterase (PDE) activity in homogenates of the smooth muscle of guinea-pig ileum

	Cyclic AMP-PDE	Cyclic GMP-PDE
AH 21-132	5.08 ± 0.17	$4.19 \pm 0.30^*$
Theophylline	3.41 ± 0.09	3.21 ± 0.11

Data are mean values of $-\log$ molar $\text{IC}_{50} \pm \text{s.e.mean}$ derived from 6 experiments. * indicates a significant ($P < 0.05$) difference between mean values of $-\log$ molar IC_{50} for cyclic AMP- and cyclic GMP-dependent phosphodiesterase.

Cyclic nucleotide phosphodiesterase (PDE) inhibition and its relation to the intestinal smooth muscle relaxant action of AH 21-132

AH 21-132 has been reported to act as a competitive inhibitor of cyclic AMP-PDE isolated from rat brain (Markstein *et al.*, 1984) and in guinea-pig trachealis to exhibit selectivity as an inhibitor of cyclic AMP-dependent PDE as opposed to cyclic GMP-

dependent PDE (Small *et al.*, 1989). The present observation that AH 21-132 acts as an inhibitor of PDE derived from the smooth muscle of guinea-pig ileum and exhibits selectivity for cyclic AMP-PDE as opposed to cyclic GMP-PDE is consistent with the findings made in rat brain and guinea-pig trachealis.

The concentrations ($>10\mu\text{M}$) of AH 21-132 required to exert spasmolytic effects in guinea-pig ileum were found to be very similar to those causing marked inhibition of PDE activity in homogenates of the ileal smooth muscle. This allows the possibility that PDE inhibition may be involved in the relaxant action of AH 21-132.

The $-\log \text{IC}_{50}$ values for AH 21-132 as an inhibitor of cyclic AMP-PDE and cyclic GMP-PDE derived from the ileum (Table 2) were almost identical to those described for the corresponding enzymes of guinea-pig trachealis (Small *et al.*, 1989). This suggests that tissue-related differences in the potency of AH 21-132 either as an inhibitor of cyclic AMP-PDE or an inhibitor of cyclic GMP-PDE (as measured with $1\mu\text{M}$ cyclic nucleotide as substrate) do not account for the difference (see above) in the potency of AH 21-132 in suppressing the spontaneous tone of guinea-pig trachea compared with its potency as a relaxant of intestinal smooth muscle. Of course, the present measurements of phosphodiesterase activity were performed using crude tissue extracts and a single nucleotide concentration. Accordingly they do not discriminate between the various isozymes of PDE (Torphy, 1988) now known to exist. It may be that AH 21-132 exhibits selectivity as an inhibitor of a PDE isozyme that plays an important role in the mechanism by which relaxation is achieved and which exhibits differing distribution or activity between airways and gastrointestinal smooth muscle.

An alternative explanation of the low potency of AH 21-132 in relaxing intestinal smooth muscle may be that (in contrast to its action on airways smooth muscle) such relaxant activity is not founded on its ability to inhibit cyclic nucleotide-dependent PDE. In any event, were AH 21-132 to receive clinical use

as a bronchodilator, its low potency in relaxing intestinal muscle *in vitro* may be predictive that inhibition of intestinal motility would not feature as an unwanted effect.

Antagonism at prejunctional receptors for adenosine

Sawynok & Jhamandas (1976) showed that adenosine could suppress the twitches of transmurally stimulated guinea-pig ileum by preventing ACh release and that this action could be antagonized by theophylline. Using a range of agonists at adenosine receptors, Paton (1981; 1988) has obtained evidence which indicates that the adenosine receptor which modulates ACh release in the ileum is of the A_1 subtype. Accordingly it may be suggested that theophylline prevents adenosine-induced ileal twitch suppression by acting as an antagonist at A_1 receptors for adenosine.

The ability of theophylline to inhibit adenosine-induced ileal twitch suppression was confirmed in the present study. However, since AH 21-132 did not share this action of theophylline, we conclude that AH 21-132 does not act as an antagonist at A_1 receptors for adenosine. Our findings are consistent with the observation (Markstein *et al.*, 1984) that AH 21-132 did not inhibit the binding of $[^3\text{H}]\text{-N}^6$ -cyclohexyladenosine to synaptosomes of rat brain. It is also of relevance to the possible therapeutic use of AH 21-132 in the therapy of asthma. A number of the unwanted effects of theophylline observed during its use for the treatment of asthma have been ascribed to its ability to act as an antagonist at adenosine receptors (Persson, 1985; Jenne, 1987). The results of the present experiments suggest that AH 21-132 might lack many of the unwanted effects of theophylline which are attributable to blockade of adenosine receptors.

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