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Trials of the bronchodilator activity of the isoenzyme-selective phosphodiesterase inhibitor AH 21-132 in healthy volunteers during a methacholine challenge test

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1 An approximately steady-state reduction of specific airway conductance was induced in normal human subjects by means of a methacholine individualized loading + maintenance dose regime. Tested against this background bronchoconstriction, the mixed type III/IV phosphodiesterase inhibitor AH 21-132, ingested in doses up to 90 mg, had no detectable bronchodilator activity.

2 AH 21-132, infused intravenously over 15 min, evoked short-lived bronchodilatation at doses of 20 and 40 mg, without affecting blood pressure or heart rate.

3 AH 21-132, mixed 1:18.5 by weight with sucrose, dissolved in saline, nebulized and inhaled in doses between 2 and 24 mg of AH 21-132, produced dose-dependent bronchodilatation. The ED_{50} was estimated as 9.2 mg AH 21-132. The peak relief of imposed bronchoconstriction was 80% and the apparent half-time of removal of AH 21-132 from its site of action was 25 min.

4 Inhaled, nebulized, hypertonic sucrose had a minor bronchodilator action.

5 AH 21-132, by intravenous and inhaled routes of administration, provides relief of methacholine-induced bronchoconstriction.

Keywords AH 21-132 bronchodilators methacholine normal human subjects sucrose

Introduction

AH 21-132 (Markstein *et al.*, 1984) is a benzonaphthyridine derivative ((±)cis-6-(*p*-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo-[c] [1,6]naphthyridine) that relaxes airways smooth muscle *in vitro* with many features reminiscent of alkylxanthines (Boyle *et al.*, 1989a; Small *et al.*, 1989a). Bewley & Chapman (1988) found AH 21-132 to be spasmolytic to the airways of ventilated guinea-pigs after either intravenous or intraduodenal administration.

The actions of AH 21-132 on smooth muscle can be discriminated from those of alkylxanthines in five respects. 1) AH 21-132 is approximately 35 times more potent than theophylline on airways smooth muscle *in vitro* and 10 times more potent *in vivo*. 2) AH 21-132 is selective for airways compared with some other kinds of smooth muscle (Small *et al.*, 1989a,b). This is an unusual finding that suggests a possible role for the drug as a bronchodilator. 3) Unlike theophylline, AH 21-132 does not antagonize adenosine in depressing the twitches elicited by electrical transmural stimulation in longitudinal smooth muscle of guinea-pig ileum (Small *et al.*, 1989b). 4) Removing the airways epithelium potentiates AH 21-132 but not aminophylline or theophylline (Bewley & Chapman, 1988; Boyle *et al.*, 1989a; Small *et al.*, 1989a). 5) AH 21-132 does not share the ability of alkylxanthines to release Ca^{2+} from intracellular stores in airways smooth muscle (Boyle *et al.*, 1989a; Small *et al.*, 1989a).

AH 21-132 inhibits competitively cyclic AMP phosphodiesterase (PDE) in rat brain (Markstein *et al.*, 1984) and in guinea-pig trachealis and ileal smooth muscle (Boyle *et al.*, 1989a,b; Small *et al.*, 1989a,b). It inhibits selectively the type III and type IV isoenzymes derived from homogenates of guinea-pig cardiac ventricles and bovine trachealis (Berry *et al.*, 1989; Elliott *et al.*, 1991; Giembycz & Barnes, 1991; Giembycz *et al.*, 1989). Consequential cAMP accumulation and protein kinase A activation were also demonstrated (Giembycz & Barnes, 1991).

The concentration range over which AH 21-132 inhibits the hydrolysis of cAMP by homogenates of

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guinea-pig and bovine trachealis encompasses that required to depress the spontaneous mechanical tone of the tissue and cause quite small amounts of spasmolysis and anti-spasmogenic effect against methacholine (MeCh) (Boyle *et al.*, 1989a; Giembycz & Barnes, 1991; Small *et al.*, 1989a). The smooth muscle relaxant and cAMP phosphodiesterase inhibitory activity reside mainly in the (−)-enantiomer (Small *et al.*, 1991).

After intravenous infusion of platelet activating factor or isoprenaline, the airways of guinea pigs become hyper-responsive to intravenous bombesin (Kristersson *et al.*, 1988). This hyper-responsiveness is inhibited by AH 21-132, which suggests that AH 21-132 may be useful as a prophylactic agent in asthma. This preventative role is in addition to but independent of any bronchodilator activity.

This paper asks the question, does AH 21-132 display bronchodilator activity in man? Preliminary reports of some of these data have been made (Foster & Rakshi, 1990a,b).

Methods

Three trials were conducted to assay the potency and effectiveness of AH 21-132 as a bronchodilator, when administered by the oral, intravenous and inhaled routes.

Measurement of airway conductance

A whole body plethysmograph of the pressure-corrected volume displacement kind (Pulmorex; Fenyves & Gut) was used essentially as described by Tattersfield & Keeping (1979). On each occasion that a subject visited the plethysmographic recording chamber, 2 min was allowed for thermal equilibration. Then, over 2 to 4 min, trained subjects panted on command with a consistent rate, depth and rhythm starting from functional residual capacity. Five technically-satisfactory X-Y plotter recordings were made of each of the V_{tg} slope and Gaw loop.

The distribution of sGaw deviates from normal and shows non-uniformity of variance (heteroscedasticity) —pitfalls in parametric statistical analysis. The logarithmic transformation establishes homoscedasticity and also improves the goodness of fit to a normal distribution (Foster *et al.*, 1991; Guyatt *et al.*, 1970). For these reasons results are presented in terms of $\log_{10}$ specific airway conductance ($\log_{10}$ sGaw).

Subjects

Fifteen normal subjects (fourteen male) aged 21–58 years (median 23.5) and within 15% of ideal bodyweight for height and frame size were studied. Each subject received a complete medical assessment. This included: full blood count and white cell differential count, blood urea and electrolyte concentrations, liver function tests, chest X-ray and electrocardiogram. Volunteers were excluded on history of asthma or atopy, medication with any drug, history of smoking, receipt of any other investigational drug within the previous 12 weeks, history of respiratory tract infection or active immunization within the previous 6 weeks, history of aspirin consumption within the previous 2 weeks, evidence of hepatic, renal or cardiac dysfunction, evidence of blood eosinophilia, positive serology for HIV or hepatitis B and the presence of metabolites of drugs of abuse in the urine.

Ethical considerations

Each subject gave written, informed consent to each study and the studies were approved by the Clinical Research Ethics Committee of the Central Manchester Health Authority.

General features of studies

Each subject attended at the same time of day, having consumed only a light meal. Subjects abstained from imbibing alcohol and caffeine-containing drinks for the previous 24 and 12 h respectively. AH 21-132 administered by the oral, intravenous or inhaled routes was the subject of bioassay for bronchodilator activity in normal volunteers. Such bioassay was rendered possible by the use of inhaled, nebulized MeCh to provide a highly reproducible, near-constant, background reduction in airway conductance as described by Foster *et al.* (1991). Inhaled drugs were delivered by an air compressor (Medix Ltd, Compact AC) driving a Bard jet nebulizer. MeCh dosage individualization was performed on each volunteer over three sessions as described by Foster *et al.* (1991). sGaw measurements were made 8 min after a 2 min inhalation of nebulized 0.9% saline (baseline sGaw) or MeCh. The initial loading dose of MeCh was sufficient to cause a 65–75% reduction in baseline sGaw and then subsequent maintenance doses of MeCh were inhaled at regular intervals to maintain this level of bronchoconstriction. All inhaled doses quoted represent the dose inspired, which was taken as one third of that nebulized.

The trial of AH 21-132 by the oral route

This trial sought to establish an efficacious oral bronchodilator dose of AH 21-132 (subject to a limit of 90 mg). It was a randomized, double-blind, placebo-controlled, multiple-dose, cross-over study in 12 male volunteers. Each subject received 9, 30 and 90 mg oral doses of AH 21-132 and a placebo in a random order, with a minimum of 72 h between each dosage. Fifty minutes after dosing, subjects inhaled the loading dose of MeCh to induce bronchconstriction followed by their predetermined maintenance doses over the next 4 h. Measurements of blood pressure and pulse rate were made before ingestion of the study medication and then after inducing bronchoconstriction at 30 min intervals to 2 h and then hourly to 4 h. Measurements of sGaw were made before drug ingestion, at 20 min intervals during the first 2 h of the MeCh-induced bronchoconstriction and thereafter at 30 min intervals to 4 h.

The trial of AH 21-132 by the intravenous route

This trial sought to establish an efficacious intravenous bronchodilator dose of AH 21-132 (subject to a limit of 80 mg). It was a randomized, open (because the solution of AH 21-132 is coloured yellow), placebo-controlled, cumulative dose, cross-over study in 12 volunteers, with a minimum of 72 h between each limb of the trial.

The baseline sGaw, blood pressure and pulse rate were measured twice before subjects inhaled the loading dose of MeCh to induce bronchoconstriction followed by their predetermined maintenance doses at 15 min intervals to 3.5 h. The onset of infusion of the study medication occurred after the first MeCh maintenance dose. Each subject received cumulative intravenous doses of 5, 5, 10, 20 and 40 mg of AH 21-132 over 15 min incrementing every 30 min or similar volumes of a saline placebo in a random order. Measurements of sGaw, blood pressure and pulse rate were made before first infusion of the study medication and then every 15 min to 3.75 h.

The trial of AH 21-132 by the inhaled route

This trial sought to establish the tolerability, time course of onset and offset of effect and an efficacious bronchodilator dose of inhaled nebulized AH 21-132 (subject to a limit of 32 mg). It was an open (because the solution of AH 21-132 is coloured yellow) cross-over study in 12 volunteers.

Tolerability and time course of effect The tolerability of inhaled AH 21-132 was assessed by the subjects on a discomfort scale of 0 (none) to 3 (severe/intense) both before and 5 and 30 min after the inhalation of a single 12 mg dose of AH 21-132. The following symptoms were each assessed: nasal itching, pain or soreness, odour, taste and nausea. The operator also assessed signs of each of nasal and oral erythema, oedema or bleeding, rhinorrhoea and cough on the same 0–3 scale.

In the time course study, after measurements had been made of baseline sGaw, blood pressure and pulse rate, subjects inhaled the loading dose of MeCh to induce bronchoconstriction followed by the appropriate maintenance doses at 15 min intervals to 3 h. A single nebulized dose of 12 mg AH 21-132 was inhaled 5 min after the first maintenance dose and sGaw, blood pressure and pulse rate were measured every 15 min to 3.25 h.

Dose-effect study In this study a similar protocol was followed except that doses of AH 21-132 of 2, 6 and 24 mg were inhaled at 30 min intervals. These doses were administered over 2, 11 and 21 min respectively. Similar volumes of saline were used as a placebo and a minimum of 72 h was allowed between each limb of the trial. As before, measurements of sGaw, blood pressure and pulse rate were made before first inhalation of AH 21-132 and then every 15 min to 3.25 h.

The effect of sucrose by the inhaled route

These experiments imitated the protocols of the trials of AH 21-132 by the inhaled route, using four volunteers (three had been subjects in the previous trial). The same doses and concentrations of sucrose were used as in the previous studies with inhaled AH 21-132. For the time course study the dose was 222 mg. For the dose-effect study the doses were 37, 111 and 444 mg.

Statistical treatment

Repeated measures analysis of variance, using the MANOVA routine of the SPSS package (Norusis, 1988), was used to assess the null hypotheses that:

1 the baseline $\log_{10}$ sGaw values, measured before any bronchoconstrictor drug was inhaled, did not differ
2 the $\log_{10}$ sGaw values, lowered by inhalation of the loading and first maintenance doses of MeCh, and measured before any bronchodilator drug was administered, did not differ
3 the series of $\log_{10}$ sGaw values, representing the bronchoconstriction imposed by the repeated maintenance doses of MeCh in the absence of AH 21-132 (placebo treatment), showed no consistent trend with time
4 the $\log_{10}$ sGaw values after AH 21-132 were not different from those after placebo. Comparisons were made between single $\log_{10}$ sGaw values and also between the means of small groups of $\log_{10}$ sGaw values.

Both time and drug effects were treated as within-subject variables.

Results

Oral AH 21-132

Figure 1 shows the mean changes in $\log_{10}$ sGaw induced in the 12 volunteers by the loading and maintenance doses of MeCh on the four study days differentiated by the dose of oral AH 21-132 taken at time zero. The loading dose of MeCh (2.0 [1.6, 2.4 geometric mean—s.e. mean, mean + s.e. mean] mg) produced a 58% reduction in baseline sGaw. In the placebo control experiment, MeCh at the maintenance dose rate (0.025 ± 0.002 of the loading dose min^{-1}) produced a 65% reduction in baseline sGaw. There were no significant differences (drug effect $P = 0.67$, time by drug interaction $P = 0.18$) between the results on study days when AH 21-132 was taken and those when placebo was taken. Similarly there were no significant differences in blood pressure or pulse rate results between active drug and placebo study days.

Intravenous AH 21-132

Figure 2 shows the mean changes in $\log_{10}$ sGaw induced in the 12 volunteers by the loading and maintenance doses of MeCh on the two study days differentiated by the infusion of a cumulative series of doses of AH 21-132 or placebo. The pooled baseline $\log_{10}$ sGaw was -0.662 ± 0.017. The two mean baseline values did not differ significantly ($P = 0.88$). The loading dose of MeCh (2.0 [1.5, 2.7 geometric mean—s.e. mean, mean + s.e. mean] mg) reduced $\log_{10}$ sGaw to -1.109 ± 0.023

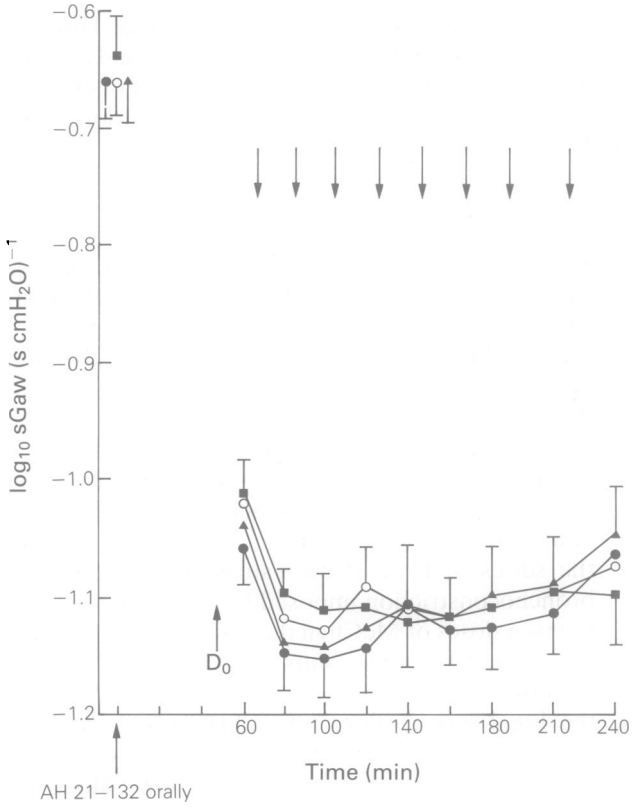

Figure 1 The effect of orally administered AH 21-132 on the establishment and maintenance of a steady-state reduction in specific airway conductance by MeCh.

After an initial estimate of sGaw had been made, AH 21-132 or placebo was taken orally. 50 min later the individualized loading dose of nebulized MeCh was inhaled (at the symbol D_0) and individualized maintenance doses were inhaled at 20 (later 30) min intervals (at the downward-pointing arrows).

The vertical axis represents the $\log_{10}$ specific airway conductance. The plotted points are means, and the bars s.e. mean, of the results in 12 subjects. ○ placebo; ▲ 9 mg, ● 20 mg, ■ 90 mg of AH 21-132.

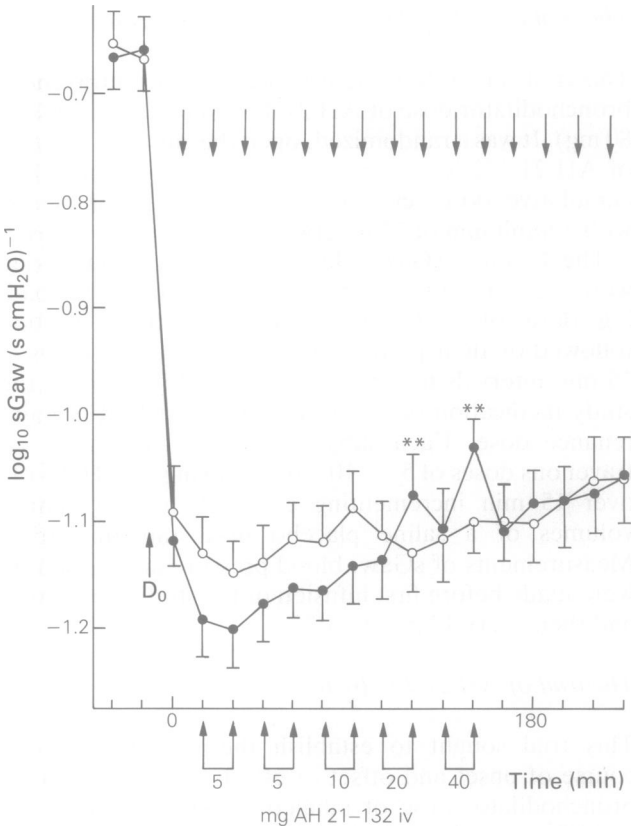

Figure 2 The effect of intravenously infused AH 21-132 on the maintenance of a steady-state reduction in specific airway conductance by MeCh.

After two initial estimates of sGaw had been made the individualized loading dose of nebulized MeCh was inhaled (at the symbol D_0) and individualized maintenance doses were inhaled at 15 min intervals (at the downward-pointing arrows).

Each dose of AH 21-132 (●) or placebo (○) was infused over 15 min at 30 min intervals.

The vertical axis represents $\log_{10}$ specific airway conductance. The plotted points are means, and the bars s.e. mean, of the results in 12 subjects. $^{**}P < 0.005$.

(a 64% reduction in baseline sGaw). In the placebo control experiment, MeCh at the maintenance dose rate (0.026 ± 0.002 of the loading dose min^{-1}) produced a mean $\log_{10}$ sGaw of -1.141 ± 0.035 (a 67% reduction in baseline sGaw). In the placebo control experiment the relationship between $\log_{10}$ sGaw and time had a slope of 0.00034 ± 0.00008 min^{-1}. There was a just significant difference ($P = 0.05$) between mean log sGaw in the two experiments after the first maintenance dose of MeCh (immediately before intravenous drug administration).

There were significant differences between the $\log_{10}$ sGaw results on study days when AH 21-132 was infused and those on days when placebo was infused. Significant bronchodilatation was detected at the fourth and fifth AH 21-132 doses (time by drug interaction $P < 0.001$) representing the cumulative administration of 40 and 80 mg respectively. There were no significant differences in blood pressure or pulse rate results between active drug and placebo study days.

Inhaled AH 21-132

Figure 3 shows the effect of AH 21-132 on $\log_{10}$ sGaw in 12 volunteers. AH 21-132 was given by inhalation as either a single dose or a cumulative series of doses.

There were few significant scores in the tolerability study at 5 or 30 min but during the first 30 s to 3 min after the beginning of inhalation most (11/12) subjects experienced bouts of mild to moderate cough. 4/12 of the subjects reported mild warmth, itching or irritation in the nose, throat or chest at 5 min and 4/12 reported a mild bitter taste extending to 30 min.

The pooled baseline $\log_{10}$ sGaw was -0.717 ± 0.015; the three mean baseline values did not differ significantly ($P = 0.69$). The loading dose of MeCh (2.2 [1.7, 2.8 geometric mean − s.e. mean, mean + s.e. mean] mg) reduced $\log_{10}$ sGaw to -1.113 ± 0.023 (a 62% reduction in baseline sGaw). In the placebo control experiment the maintenance dose rate (0.025 ± 0.002 of the loading dose min^{-1}) produced a mean $\log_{10}$ sGaw of -1.200 ± 0.029 (a 68% reduction in baseline sGaw). In the placebo experiment the relationship between $\log_{10}$ sGaw and time had a slope of 0.00036 ± 0.00017 min^{-1}. There was no significant difference ($P = 0.15$) between mean $\log_{10}$ sGaw in the three experiments after the first maintenance dose of MeCh (immediately before first inhalation of study medication).

In the time course study (Figure 3a) analysis of variance between times 15 and 90 min showed each of

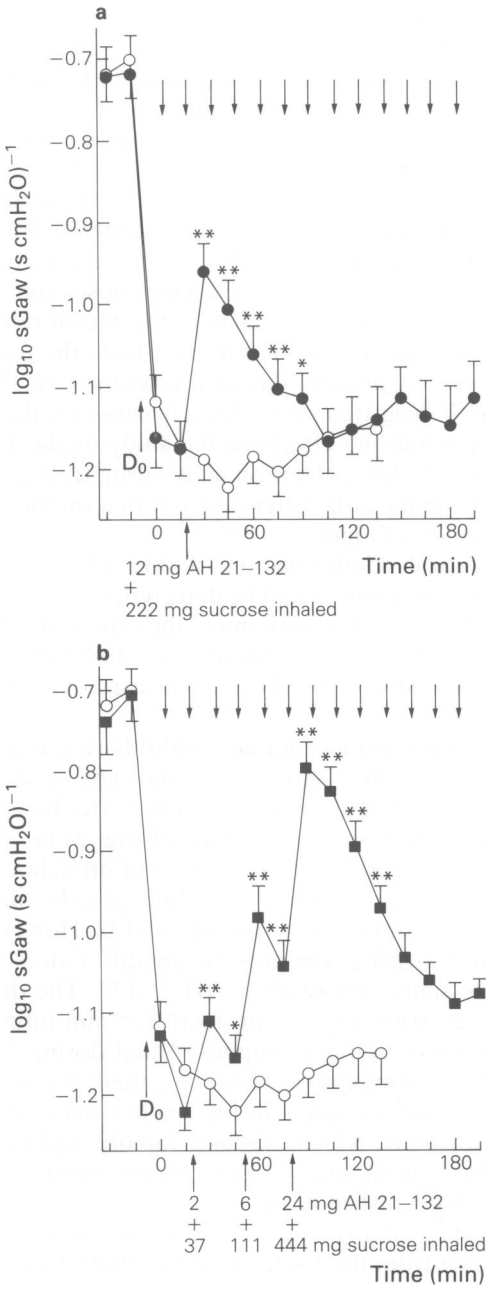

Figure 3 The effect of inhaled nebulized AH 21-132 on the maintenance of a steady-state reduction in specific airway conductance by MeCh. (a) Time course of effect (○ saline, • AH 21-132 12 mg mixed with sucrose 222 mg in saline). (b) Dose dependency of effect (○ saline—the same data as in (a), ■ AH 21-132 mixed with sucrose in saline).

After two initial estimates of sGaw had been made the individualized loading dose of nebulized MeCh was inhaled (at the symbol D_0) and individualized maintenance doses were inhaled at 15 min intervals (at the downward-pointing arrows).

The vertical axis represents $\log_{10}$ specific airway conductance. The plotted points are means, and the bars s.e. mean, of the results in 12 subjects. $^{**}P < 0.005$; $^{*}0.005 < P < 0.05$.

the main effects of time and drug and their interaction to be highly significant ($P < 0.001$). There was a significant increase (size 0.21) in $\log_{10}$ sGaw 12 min after the beginning of inhalation of AH 21-132 12 mg (mixed with 222 mg sucrose) representing a 46% relief of the imposed bronchoconstriction. The effect then declined in approximately linear fashion over 75 min with an offset slope of -0.00278 ± 0.00026 min^{-1}.

In the cumulative log dose-effect study (Figure 3b) analysis of variance between times 15 and 105 min showed each of the main effects of time and drug and their interaction to be highly significant ($P < 0.001$). There was a significant increase of $\log_{10}$ sGaw 12 min after the beginning of inhalation of each of the doses. The size of the effect was an approximately linear function of the $\log_e$ of the cumulative dose (slope 0.113 ± 0.018). After the highest dose of 24 mg (cumulative 32) AH 21-132 combined with 444 mg (cumulative 592) sucrose, relief reached 80% of the imposed bronchoconstriction. The ED_{50} was 9.2 (95% C.I. 6.4, 13.6) mg AH 21-132 combined with 170 (118, 252) mg sucrose.

After the development of the peak effect, the effect declined in approximately linear fashion over 90 min with an offset slope of -0.00355 ± 0.00026 min^{-1}. The slope of the offset curve obtained by pooling data from this study and the time course study was -0.0032 ± 0.0004 min^{-1}. The elimination rate constant of the drug from its site of action can be estimated from the ratio between this and the slope of the $\log_e$ dose-effect curve (Foster *et al.*, 1991). It was found to be 0.028 min^{-1}, giving a half time for this process of 25 min.

There were no significant differences in blood pressure or pulse-rate results between active drug and placebo study days.

Inhaled sucrose

Figure 4 shows the mean changes in $\log_{10}$ sGaw induced in four volunteers by the loading and maintenance doses of MeCh on the 3 study days. Sucrose and placebo were inhaled either as single doses or as cumulative series of doses.

The pooled baseline $\log_{10}$ sGaw was -0.665 ± 0.027; the three mean baseline values did not differ significantly ($P = 0.69$). The loading dose of MeCh (1.2 [0.5, 3.0 geometric mean − s.e. mean, mean + s.e. mean] mg) reduced $\log_{10}$ sGaw to -1.253 ± 0.042 (a 74% reduction in baseline sGaw). In the placebo control experiment, MeCh at the maintenance dose rate (0.027 ± 0.003 of the loading dose min^{-1}) produced a mean $\log_{10}$ sGaw of -1.283 ± 0.061 (a 77% reduction in baseline sGaw). In the placebo experiment the relationship between $\log_{10}$ sGaw and time had a slope of 0.00047 ± 0.00025 min^{-1}. There was a significant difference ($P = 0.026$) between mean log sGaw in the three experiments after the first maintenance dose of MeCh (immediately before the sucrose was inhaled).

In the time course study (Figure 4a) analysis of variance between times 15 and 90 min revealed that neither the main effect of sucrose ($P = 0.13$) and time ($P = 0.1$) nor their interaction ($P = 0.056$) were significant.

In the cumulative log dose effect study (Figure 4b) analysis of variance between times 15 and 105 min showed that the main effect of sucrose ($P = 0.66$) was not significant but the time main effect ($P = 0.003$) and sucrose by time interaction ($P = 0.002$) were. There was a nearly significant ($P = 0.052$) increase of $\log_{10}$ sGaw 12 min after the beginning of inhalation of the highest dose.

In the three subjects who received both AH 21-132 mixed with sucrose and sucrose alone on two occasions, the effect of the former was always larger than

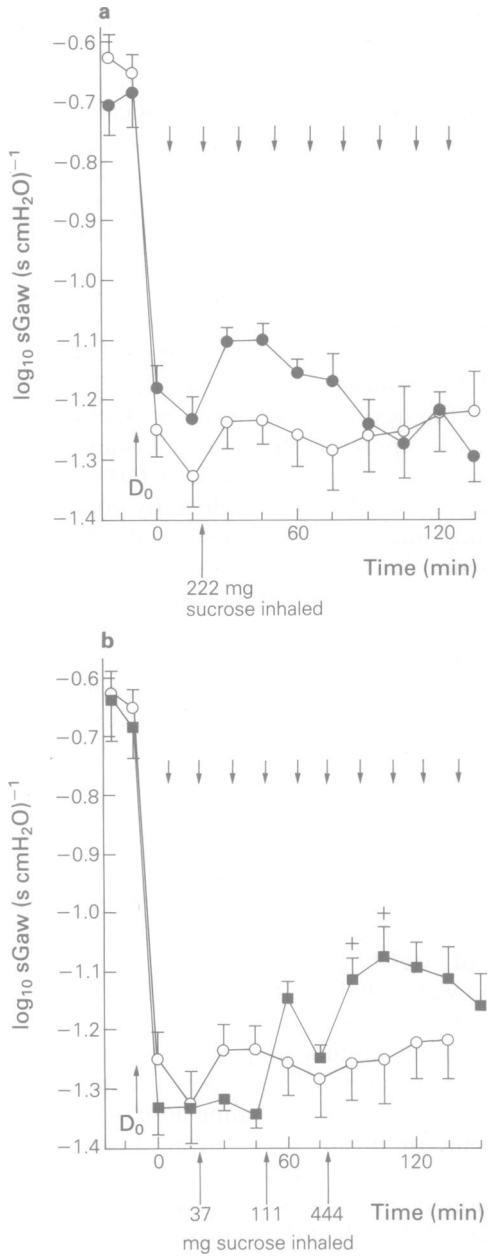

Figure 4 The effect of inhaled nebulized sucrose on the maintenance of a steady-state reduction in specific airway conductance by MeCh. (a) Time course of effect (○ saline, ● sucrose 222 mg in saline). (b) Dose dependency of effect (○ saline—the same data as in (a), ■ sucrose in saline).

After two initial estimates of sGaw had been made the individualized loading dose of nebulized MeCh was inhaled (at the symbol D_0) and individualized maintenance doses were inhaled at 15 min intervals (at the downward-pointing arrows).

The vertical axis represents specific airway conductance as $\log_{10}$ sGaw. The plotted points are means, and the bars s.e. mean, of the results in 12 subjects. + = $P \approx 0.05$.

that of the latter − mean difference 0.179 ± 0.018, $P = 0.0002$, judged at the 12 mg AH 21-132 + 222 mg sucrose dose level.

Discussion

The principal objective of the trials, namely to reveal and quantify any bronchodilator activity of AH 21-132 in man has, within certain limits, been achieved.

The maintained bronchoconstriction produced on the placebo treatment day of each trial by repeated maintenance dosing with MeCh proved to be not quite constant. After a peak of reduction of sGaw close to the target size at or shortly after the time of the first maintenance dose, there was a small, slow, but significant decline for the remainder of the observation period. This decline did not deviate significantly from a linear trend. Whether this represents a consistent under-estimation of the maintenance dose rate required or a developing tachyphylaxis to the MeCh remains to be determined. It does not detract from the use of the results on the placebo day as a reference for those on the active drug treatment days, because by the end of the day, when the effects of the study medication had worn off, the $\log_{10}$ sGaw on active drug treatment days was not significantly different from that on the placebo treatment day (Figures 2, 3a).

After oral administration of AH 21-132 no bronchodilator response could be detected, even at a dose of 90 mg. This does not undermine the reported selectivity of the drug for airways (Small *et al.*, 1989a,b), because there was also no evidence of cardiovascular disturbance.

After intravenous administration there was evidence of bronchodilator activity without any evidence of alterations in blood pressure or heart rate. It is possible that the responses to 20 and 40 mg were artefacts resulting from the different degrees of bronchoconstriction prevailing on active drug and placebo days just prior to the administration of AH 21-132. However, this is unlikely because the pre-existing differences were of opposite sign to those after AH 21-132. The design of the intravenous infusion protocol (15 min infusions at 30 min separation) had supposed that dosing could be regarded as cumulative. However, there was a striking brevity to the increases in $\log_{10}$ sGaw associated with these infusions, which suggests rapidly declining concentrations at the site of action on cessation of infusion, perhaps by redistribution.

The clearest evidence of bronchodilator effectiveness arose from the local administration of the drug to the airways by inhalation. It was no surprise, in view of the previous results, the doses employed and the route of administration, that no disturbance of cardiovascular function accompanied the bronchodilatation. The increase of $\log_{10}$ sGaw showed a clear time-dependent and dosage-dependent association with drug inhalation. It developed quickly, was always maximal at the first measurement time after initiation of the inhalation and then waned in an approximately linear fashion. This implies an exponential decline in drug concentration at its site of action. The half-time of this decay was estimated as 25 min (Foster *et al.*, 1991).

The potency of the inhaled drug could be estimated from the dose causing 50% relief of the imposed bronchoconstriction. This was estimated to be 9.2 mg AH 21-132. The effectiveness could be estimated from the peak magnitude of relief achievable. This averaged 80% relief of the imposed bronchoconstriction and there was no trend in the dose-effect data to suggest that this was the maximal effect.

One problem of interpretation that must be addressed arises from the presence of sucrose as an excipient

in the formulation of AH 21-132 for inhalation. The presence of sucrose was revealed to us at a late stage in the conduct of these trials. We felt it essential to attempt to control for that presence in the hope that any bronchodilator result of the trial could then be correctly attributed to AH 21-132. We employed three of the subjects of the inhaled trial and one new one.

Sucrose, at the highest dose employed, did seem to cause a bronchodilatation. Kirkpatrick *et al.* (1980) have demonstrated on bovine airway smooth muscle *in vitro* that the direct effect of sucrose, as well as other hyperosmolar solutions, is an active contraction in addition to cellular shrinkage. In view of the distance between the mucosal site of deposition of inhaled droplets and the bronchial smooth muscle and the turnover of the intervening submucous interstitial fluid, it is questionable whether such direct osmotic effects on the smooth muscle could be demonstrated *in vivo*. It seems more likely, in our situation, that any effects exerted are on the mucosal cells. This might be shrinkage with consequent passive increase in airway conductance but an indirect and more active process based upon release of a bronchial smooth muscle relaxant factor from airway mucosal cells by a hyperosmotic stimulus is also a possibility (Munakata *et al.*, 1988). The important contrast in our experimental results is that between the small size of the effect of sucrose alone and the large size of the effect of the mixture of AH 21-132 and sucrose. Hence, it is likely that AH 21-132 does indeed exert effects of approximately the potency and effectiveness described. This conclusion must be qualified by the proviso that, should there be a synergism (rather than addition) between AH 21-132 and sucrose, then our results would overestimate the potency and effectiveness of the AH 21-132 component.

The spasmolytic action of AH 21-132 that is here demonstrated represents a functional antagonism of MeCh. It is presumed to be contingent upon the inhibition of type III/IV PDE in airway smooth muscle cells but the exact linkage between these events remains speculative. A more direct interaction was sought by Giembycz *et al.* (1990), who measured effects of AH 21-132 on MeCh-induced inositol phosphate formation in bovine tracheal smooth muscle. They found that phosphoinositide-specific phospholipase C could be inhibited by AH 21-132 but only at concentrations much exceeding those causing maximal spasmolytic and antispasmodic effects.

The use of MeCh spasm to provide the baseline from which spasmolysis is measured makes the present model specifically effective in detecting and quantifying the bronchodilator activity of drugs. It must be recalled that other activities in the drug or the expression of the same activity (PDE inhibition) in other cell types (e.g. Kristersson *et al.*, 1988) might contribute to or even dominate any anti-asthma action that it may possess.

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