Comparison of the effects of salbutamol and adrenaline on airway smooth muscle contractility in vitro and on bronchial reactivity in vivo

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Abstract

Background — The effect of adrenergic agonists in asthma depends on their net effect on microvascular leakage, mucosal oedema, vascular clearance of spasmogens, inhibition of cholinergic neurotransmission, and airway smooth muscle contractility. It has been postulated that adrenaline, by virtue of its alpha effects on the vasculature and cholinergic neurotransmission, may have additional useful properties in asthma compared with selective beta agonists such as salbutamol.

Methods — The airway effects of adrenaline (a non-selective adrenoreceptor agonist) were compared with the selective beta agonist salbutamol. Their airway smooth muscle relaxant potencies and effect on histamine contraction in human bronchi in vitro were compared with their effects on airway calibre and histamine reactivity in asthmatic subjects in vivo. For the in vitro studies changes in tension were measured in response to these agents in thoracotomy specimens of human airways. In vivo the effects of adrenaline and salbutamol on airway calibre and histamine reactivity were measured in eight subjects with mild to moderate asthma in a randomised crossover study.

Results — Salbutamol and adrenaline had approximately equivalent airway smooth muscle relaxant potencies in vitro and bronchodilator potency in vivo. However, their effects on histamine induced contraction in vitro were significantly different from their effects on histamine reactivity in vivo. Salbutamol was less potent in vitro producing a mean (SE) 2.4 (0.15) doubling dose increase in the histamine EC50 and adrenaline a 5.2 (0.18) doubling dose increase (mean difference between salbutamol and adrenaline 2.8 doubling doses; 95% CI 1.1 to 4.5). Salbutamol had no effect on the maximal response to histamine whereas adrenaline reduced it by 54%. In contrast, salbutamol was more potent in vivo producing a mean (SE) increase in PD20 histamine of 1.84 (0.5) doubling doses whereas adrenaline was without effect increasing PD20 by only 0.06 (0.47) doubling doses (mean difference between adrenaline and salbutamol 1.78, 95% CI 0.26 to 3.29 doubling doses).

Conclusions — These findings suggest that the alpha adrenergic airway effects of non-selective adrenoreceptor agonists such as adrenaline offer no additional protection against histamine-induced bronchoconstriction in vivo than beta2 selective drugs such as salbutamol, despite adrenaline providing greater protection against histamine-induced contraction in vitro. The differences between the effects of these agents in vitro and in vivo may be related to their opposing vascular effects in vivo.

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Beta2 adrenergic agonists are extensively used as bronchodilators in the treatment of asthma and chronic obstructive airways disease. These drugs were developed to avoid the cardiac toxicity associated with less selective adrenergic agents while retaining a potent relaxant effect on airway smooth muscle beta2 receptors. Several recent reviews, however, have stressed the potential importance of vascular changes in the airway in altering airway responsiveness.1,4 Adrenergic drugs designed for their effects on airway smooth muscle beta2 receptors may therefore not be the best adrenergic drugs for the treatment of asthma. Experimentally-induced vascular changes in the airways of animals and man have been shown to have significant effects on airway calibre through several interrelated mechanisms.2 Vascular congestion and/or an increase in microvascular leakage can alter mucosal thickness and mathematical modelling suggests that this can have considerable effects on bronchial responsiveness.5,6 Alpha adrenergic agonists confer protection against several thermally-induced bronchial challenges such as exercise7 and hyperventilation.8 This protection is thought to be due to the prevention of pulmonary vascular engorgement. Vascular changes may also alter bronchial responsiveness by altering the vascular clearance of constrictor mediators from the airway.9 This has been postulated for changes occurring with antigen inhalation in sheep10 and methacholine inhalation in dogs10 and man11 following treatment with vasodilators or vasoconstriction. Differences in the vascular effects of drugs may thus be very important in determining their effects on airway function.12 It has been postulated that the vascular alpha effects of adrenaline could have potential benefits
over salbutamol in the treatment of asthma.\textsuperscript{12} Non-selective adrenergic agents, through alpha-mediated vasoconstriction, might be expected to have a different effect on the bronchial and pulmonary vasculature than \beta\-agonists. For example, in an animal model of asthma microvascular leakage induced by platelet activating factor was inhibited by adrenaline, whilst the selective \beta\-agonist salbutamol had no effect.\textsuperscript{12} In acute asthma it has been shown that, whereas salbutamol produces a small fall in \textit{P}ao\textsubscript{2}, due to bronchial arteriolar dilatation and consequent redistribution of pulmonary blood to poorly ventilated areas, adrenaline produced a small but consistent rise in \textit{P}ao\textsubscript{2}, suggesting an improvement in the ventilation/perfusion relationship due to vasoconstriction.\textsuperscript{13}

The aim of our study was therefore to compare the relative potencies of adrenaline (a non-selective adrenergic agonist) and salbutamol (a \beta\-agonist) as bronchodilators and as protective agents against histamine bronchial reactivity in vivo. To determine if any difference was due to differences in their effects on airway smooth muscle we performed parallel experiments in vitro where there is little contribution from vascular changes. Surprisingly there have been no published studies directly comparing the effects of these agents on histamine-induced bronchoconstriction in human airway in vitro or on asthmatic subjects in vivo. The primary hypothesis we were testing was that adrenaline, by virtue of its alpha effects on the airways, might have additional beneficial properties compared with salbutamol on airway calibre and bronchial reactivity in asthma.

\textbf{Methods}

\textbf{IN VITRO STUDY}

Macroscopically normal human lung tissue was obtained from thoracotomy specimens of four patients (all smokers) undergoing lung resection for carcinoma and placed immediately in Krebs-Henseleit solution (KHS) of the following composition (mmol/l): NaCl 118, KCl 4.7, MgSO\textsubscript{4} 1.2, NaH\textsubscript{2}PO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2.5, NaHCO\textsubscript{3} 25, glucose 11.1, pH 7.4. Tissues were transported immediately on ice to the laboratory where they were dissected into bronchial rings and suspended under 2 g tension, in organ baths containing KHS at 37\textdegree C continuously gassed with 95\% O\textsubscript{2}/5\% CO\textsubscript{2}. No attempt was made to remove the epithelium. The tensions used had previously been shown to produce optimal, repeatable responses to histamine in preparations of a similar size. Changes in tension were recorded on four force displacement transducers (FTO3, Grass Instruments, Quincy, Massachusetts, USA) and displayed on two two-channel flat bed recorders (CR600, JF Instruments, Southampton, UK). Tissue was allowed to equilibrate under tension for one hour before each experiment.

Eight bronchial rings from each of the four subjects were studied (32 rings in total). A cumulative histamine concentration response study was performed on each ring, increasing amounts of histamine being added to each bath to produce cumulative bath concentrations over the range \textit{10}^{-7}--\textit{10}^{-9} mol/l in threefold increments. Each concentration was added at the plateau to the previous concentration. We have previously shown that the histamine concentration-response curve carried out in this way is very repeatable.\textsuperscript{14} Rings were excluded from further study if the initial control for the \textit{10}^{-5} mol/l histamine was less than 0.2 g. The tissues were then washed repeatedly over the next hour until tension had returned to baseline values. Adrenaline was then added to half of the rings to achieve a bath concentration of \textit{10}^{-5} mol/l and the maximum relaxant effect noted. This was followed by serial tenfold increases in cumulative bath concentrations up to \textit{10}^{-7} mol/l, each additional dose being given after the response had plateaued. Similar relaxation concentration-response curves for salbutamol were measured in parallel rings. Allocation of paired rings to adrenaline or salbutamol was random. The total duration of the experiment was 4-5 hours.

\textbf{Drugs}

Adrenaline and salbutamol were obtained from Sigma Chemicals, Poole, UK. Histamine acid phosphate was obtained from BDH Chemicals, Poole, UK. All drugs were dissolved in water.

\textbf{Analysis}

All contractile responses were expressed as a percentage of the initial maximal response to \textit{10}^{-4} mol/l histamine. The contractile effect of histamine was expressed as the concentration of drug producing either 20\% and 50\% of the initial maximal contraction (EC\textsubscript{20} and EC\textsubscript{50}) and the mean values before and after the drug were compared using analysis of variance. In addition, the maximum contractile responses to \textit{10}^{-4} mol/l histamine after drugs were compared by analysis of variance. The relaxant effects of salbutamol and adrenaline on inherent tone were compared at EC\textsubscript{20} and EC\textsubscript{50} values which were the concentrations of drug which produced 20\% and 50\% of maximal relaxation. The change in tension below baseline induced by adrenaline or salbutamol in these studies was expressed as a % of the tension generated by the initial histamine contraction. This standardised the response for the amount of airway smooth muscle present in each bronchial ring. The results in this study were identical whether they were expressed in this manner or in absolute terms in grams of tension. A p value of <0.05 was regarded as being statistically significant.

\textbf{IN VIVO STUDY}

\textbf{Subjects}

Eight men aged between 24 and 51 years with...
mild to moderate asthma and requiring inhaled therapy alone were studied. They were otherwise healthy and had not had a respiratory tract infection within four weeks of the first study visit. All subjects had a resting forced expiratory volume in one second (FEV₁) >60% of their predicted value and had previously demonstrated an improvement in FEV₁ of greater than 15% after 200 µg inhaled salbutamol. They also had a provocative dose of histamine causing a 20% fall in FEV₁ (PD₂₀ FEV₁, hist) of <4 µmol. All were taking inhaled β agonists either alone or with inhaled corticosteroids. Medications were continued unchanged throughout the study although β agonists were withheld six hours before each study visit. Subjects gave written consent to participation in the study which was approved by the Nottingham City Hospital ethics committee.

Measurements
FEV₁ was measured using a dry bellows spirometer (Vitalograph, Buckingham, UK) as the higher of the two successive readings within 100 ml. Histamine challenge was performed by the method of Yan et al using DeVilbiss No 40 hand-held nebulisers (DeVilbiss Co, Pennsylvania, USA). After baseline FEV₁ had been measured, subjects inhaled 0-9% saline followed by doubling doses (0-03–8 µmol) of histamine with measurement of FEV₁ 60 seconds after each inhalation. The challenge was discontinued when the FEV₁ had fallen by 20% or more from post saline values. PD₂₀ FEV₁ was estimated by linear interpolation on a log dose-response plot.

Protocol
The study had a randomised, double blind, crossover design. Subjects attended the laboratory on two non-consecutive days of the same week at the same time of day. After resting in the sitting position for 15 minutes, baseline measurements of heart rate, blood pressure, and FEV₁ were made, and a histamine challenge test performed. One hour later, provided the FEV₁ had returned to within 95% of the baseline level, subjects were asked to inhale four sequential doses (0-4, 4, 40, and 400 µg) of either salbutamol or adrenaline solutions at 10 minute intervals in a cumulative manner via an Inspiron Minineb nebuliser (Bard, Sunderland, UK). Each dose was administered in 1 ml of 0-9% sodium chloride and nebulised to dryness. Dilutions were freshly prepared in sterile 0-9% sodium chloride each day. Five and ten minutes after each dose, pulse rate, blood pressure, and FEV₁, were recorded. Ten minutes after the final dose of salbutamol or adrenaline a further histamine challenge test was performed. The aim of using incremental doses was to enable us to construct dose-response curves for the two agents.

Analysis
The PD₂₀FEV₁ hist values were log transformed before analysis and expressed as geometric mean values. The change in FEV₁ following adrenaline and salbutamol and the differences in PD₂₀FEV₁ hist between adrenaline and salbutamol were compared using Student’s paired t test. Differences in geometric mean values of PD₂₀FEV₁ between salbutamol and adrenaline were expressed in doubling doses. A p value <0.05 was regarded as significant. The study had 80% power to detect a one doubling dose change in bronchial responsiveness.

Results
IN VITRO STUDY
Thirteen paired bronchial rings gave an initial maximal contractile response of greater than 0-2 g. Six rings were rejected because they gave a response of <0.2 g.

Figure 1  Relaxant effect of increasing concentrations of salbutamol and adrenaline on inherent tone in human bronchial rings in vitro. Values shown are mean (SE) (n = 13).

Figure 2  Histamine concentration response curves before and after (A) salbutamol (10⁻⁸ mol/l) and (B) adrenaline (10⁻⁷ mol/l). Values shown are mean (SE) (n = 13).
Relaxant potency
Figure 1 shows the relaxation curves for both bronchodilators against inherent tone. The mean (SE in log units) EC20 and EC50 were 2.9 (0.16) \times 10^{-8} and 2.2 (0.07) \times 10^{-7} mol/l for salbutamol and 2.5 (0.22) \times 10^{-8} and 1.8 (0.24) \times 10^{-7} mol/l for adrenaline. No significant differences were found between salbutamol and adrenaline.

Histamine-induced contraction
Figure 2 shows the effect of the drugs on the contractile response to histamine. The mean (SE in log units) prebronchodilator histamine EC20 and EC50 values for salbutamol-treated tissues were 1.4 (0.09) \times 10^{-6} and 3.7 (0.07) \times 10^{-6} mol/l and those of adrenaline-treated tissues were 1.3 (0.05) \times 10^{-6} and 4.3 (0.06) \times 10^{-6}. There were no significant differences between the prebronchodilator values for the tissue groups treated with the two agents. Both drugs caused an increase in EC20 to histamine, and for adrenaline this was so considerable that the EC50 could not be calculated for nine of the 13 preparations. The mean (SE in log units) increase in EC20 after salbutamol was 2.4 (0.15) doubling doses. For adrenaline the mean (SE in log units) change in EC20 was 5.2 (0.18) doubling doses. The mean difference in the change in EC20 for salbutamol and adrenaline was 2.8 doubling doses with a 95% CI 1.1 to 4.5; p=0.005. At 10^{-3} mol/l histamine the mean (SE) maximum contraction as a percentage of the initial maximal response was 100.1 (14.8)% for salbutamol and 46.2 (9.0)% for adrenaline. The mean difference in maximum contraction between salbutamol and adrenaline treated tissues was 53.9% (95% CI 25.2 to 82.7), p<0.001.

IN VIVO STUDY
Bronchodilator potency
The mean (SE) FEV1 rose from 2.96 (0.26)1 to 3.41 (0.25)1 after the maximum dose of adrenaline (400 µg) and from 2.95 (0.25)1 to 3.52 (0.28)1 after the maximum dose of salbutamol (400 µg). Figure 3 shows the response of FEV1 to adrenaline and salbutamol at the different doses. Both drugs produced significant bronchodilatation at 40 µg and 400 µg when compared with baseline (p<0.001). When the bronchodilator effect of salbutamol and adrenaline were compared at each dose no significant difference was found.

Bronchial reactivity
Salbutamol produced a rise in mean (SE) PD20 histamine from 0.36 (0.13) µmol to 1.29 (0.17) µmol – that is, 1.84 doubling doses – whereas adrenaline caused a rise from 0.38 (0.10) µmol to 0.39 (0.15) µmol – that is, 0.06 doubling doses (fig 4). The difference in change in PD20 between salbutamol and adrenaline was 1.78 (95% CI 0.26 to 3.29) doubling doses, p<0.05. There were no significant changes in pulse rate or blood pressure during the study period.

Discussion
We found that the bronchodilator potencies of adrenaline and salbutamol were equivalent in asthmatic subjects in vivo. In previous studies in asthma inhaled salbutamol has been shown to produce a similar increase in FEV1 to that found in the present study when given in doses from 200 to 1000 µg.16-18 There are no previous reports of direct comparison studies of equivalent doses of inhaled salbutamol and adrenaline in asthma in vivo, previous studies differing either by the doses used16-20 or the route of administration.21 We also found that adrenaline and salbutamol were of equal potencies as airway smooth muscle relaxants in vitro with the relaxant curves virtually superimposed. Although previous studies have looked at adrenaline and salbutamol in human airway smooth muscle in vitro separately,22-25 none have directly compared the two. Studies in animal airways in vivo, however, have shown equivalent relaxant potencies of the two agents. The fact that we found a similar dose equivalence in vitro and in vivo suggests that bronchodilatation as measured acutely in vivo represents airway smooth muscle relaxation.

The most surprising finding in our study was that adrenaline had no effect on histamine reactivity in vivo, in contrast to salbutamol which produced an approximately two doubling dilution shift in histamine reactivity in vivo.
This was the opposite of what we would have expected if the alpha effects of adrenaline are important. The change we saw with salbutamol is in agreement with several other studies in asthma. The only two previous studies to look at the effect of adrenaline on histamine responsiveness in vivo looked at physiological concentrations given intravenously rather than pharmacological concentrations given by inhalation, and showed small reductions in histamine reactivity. In our present study local concentrations of adrenaline in the airway were likely to be much higher than in these studies due to the dosage and route used, and this might account for the difference by altering the ratio of $\alpha$ to $\beta$ effects. The difference in protective effect of salbutamol and adrenaline on histamine responsiveness occurred despite the drugs producing equivalent bronchodilatation. The lack of correlation between bronchodilator properties of drugs and their effects on bronchoconstriction challenges has previously been shown in studies comparing salbutamol and ipratropium.

The difference between the relative effects of adrenaline and salbutamol on histamine responses in vivo was not due to differences in their ability to inhibit smooth muscle responses to histamine. Adrenaline had a profound effect on histamine-induced contractions in vitro whereas salbutamol had little effect at a concentration producing an identical degree of smooth muscle relaxation. The histamine response curve after adrenaline was much flatter than that after salbutamol. It is difficult to draw conclusions about the significance of this without dose ranging the effect of both drugs. Although the second histamine response curve performed in vitro was from a different baseline from the first, this is analogous to the situation in vivo and was the same for both drugs. This cannot, therefore, explain the difference between drugs. Interestingly, studies in guinea pig airways in vitro have also failed to show large shifts in histamine and muscarinic agonist induced concentration-response curves after another $\beta_2$ selective drug, terbutaline. The authors inferred from their study that $\beta_2$ agonists may be protecting against histamine-induced bronchoconstriction in vivo by an effect other than their smooth muscle effects. Our findings would support this. The greater effect of adrenaline on airway smooth muscle histamine responses in vitro is most likely to be due to the fact that it is a full agonist on $\beta_2$ receptors whereas salbutamol is only a partial agonist. It is unlikely to represent an $\alpha$ effect as $\alpha$ adrenergic blockade has previously been shown to alter histamine-induced contraction in human airway smooth muscle, and although airway smooth muscle from several animal species contains $\beta_1$ receptors, it has been difficult to demonstrate them in autoradiographic studies of human airway smooth muscle. It might thus be that the partial agonist effect of salbutamol on $\beta_2$ receptors, whilst sufficient to induce relaxation, does not provide the same protection against agonist-induced airway smooth muscle contractility as a full agonist such as adrenaline. It is possible that we underestimated the effects of adrenaline in vitro due to its metabolism, but we saw no diminution of its relaxant effect over the time course of our experiment and, in any case, this would serve only to magnify the disparity between its effects in vitro and in vivo.

We have considered several explanations for the lack of effect of adrenaline on histamine responsiveness in vivo despite its marked effect on airway smooth muscle in vitro. Adrenaline has several potentially beneficial actions. Its $\beta_1$ agonist effect on airway smooth muscle would be expected to reduce histamine reactivity as it did in vitro and it would also reduce histamine-induced vasodilatation and capillary leakage through its $\alpha$ vasoconstrictor effects. The balance of its effects on the vasculature would be vasoconstrictor as it has much more potent $\alpha$ than $\beta_1$ effects.

There are three mechanisms whereby vasoactive substances might alter bronchial responsiveness. These are bronchoconstriction from a direct effect of adrenaline on airway smooth muscle. Firstly, blood flow may affect airway wall thickness through vascular engorgement; secondly, alterations in microvascular leakage may alter airway responsiveness; and thirdly, vascular changes may alter the clearance of bronchoactive substances from the airway. The net effect of these mechanisms will determine the effect of the vasoactive substance on airway reactivity.

Further evidence to support this hypothesis is that reduction of tracheobronchial blood flow in dogs prolongs methacholine-induced bronchial obstruction, and in man methacholine-induced bronchial obstruction is prevented by a potent vasodilator, prostacyclin. Similarly, allergen-induced bronchoconstriction in sensitised sheep is prolonged by the vasoconstrictor agent vasopressin, and reduced by the vasodilator nitroglycerin.

The lack of effect of adrenaline on histamine responsiveness in our study contrasts with two studies showing that the $\alpha$ agonists methoxamine and noradrenaline protect against exercise and hyperventilation-induced airflow obstruction respectively. The apparent disparity in these results may be due to the mechanisms involved in the different types of challenge tests. In bronchoconstrictor challenges with exogenous histamine where smooth muscle contraction is the main mechanism of bronchoconstriction, the major effect of a vasoconstrictor may be to reduce the vascular clearance of inhaled histamine. In contrast, in challenges such as exercise and hyperventilation where thermally-induced vasodilatation may be contributing to the airflow narrowing, the effect of a vasoconstrictor may be to protect against this. The relation between bronchial blood flow and thermally-induced bronchoconstriction may be complex, however, as volume expansion can produce contrasting effects on hyperventilation-induced broncho-
constriction depending on the timing of infusion.16

An alternative explanation for the lack of effect of adrenaline on histamine reactivity in vivo is a pharmacokinetic one, namely that adrenaline was metabolised more quickly than salbutamol. While adrenaline does undergo more rapid metabolism than salbutamol, we feel that this is a less likely explanation for its lack of effect on histamine reactivity for several reasons. We performed histamine challenges only 10 minutes after the final dose of adrenaline and these were completed in 5–10 minutes. As bronchodilatation was still maximal at the start of the histamine challenge test, it would seem unlikely that the effect would have worn off in the ensuing 5–10 minutes. Furthermore, a study by Kjellman et al 17 found no significant difference between salbutamol and adrenaline in bronchodilatation over a 50 minute period, with maximal bronchodilatation still being apparent 60 minutes after adrenaline inhalation. Precise pharmacokinetic data on the half lives of the two agents given by inhalation are, unfortunately, not available.

Finally, we considered whether the lack of effect of adrenaline on histamine responsiveness in vivo might be due to its effects on α2 receptors on cholinergic nerve terminals. 37 This would be unlikely to reduce any protective effect on histamine responsiveness.

In conclusion, salbutamol and adrenaline have similar bronchodilator potencies when given by nebulisation in asthmatic subjects in vivo and when administered directly to airway smooth muscle in vitro. Despite a greater effect on histamine-induced contractions in vitro, adrenaline was less effective in protecting histamine-induced bronchoconstriction in vivo. Our study suggests that the potential beneficial α adrenergic effects of adrenaline, such as reduced microvascular leakage and decrease in mucosal blood flow, do not protect against histamine-induced constriction in mild asthmatics when adrenaline is administered acutely by nebulisation. Furthermore, the vascular α effects of adrenaline may be antagonistic to the beneficial β2 effects, possibly by reducing vascular clearance of histamine.

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