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

David R Baldwin, Ziali Sivardeen, Ian D Pavord, Alan J Knox

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 β_2 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 EC_{20} 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 PD_{20} histamine of 1.84 (0.5) doubling doses whereas adrenaline was without effect increasing PD_{20} 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 α adrenergic airway effects of non-selective adrenoreceptor agonists such as adrenaline offer no additional protection against histamine-induced bronchoconstriction in vivo than β_2 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.

(Thorax 1994;49:1103-1108)

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 whilst retaining a potent relaxant effect on airway smooth muscle β_2 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 β 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.² This has been postulated for changes occurring with antigen inhalation in sheep⁹ 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

Respiratory Medicine Unit, City Hospital, Nottingham NG5 1PB, UK

D R Baldwin
Z Sivardeen
I D Pavord
A J Knox

Reprint requests to:
Dr A J Knox.

Received 29 November 1993
Returned to authors
4 February 1994
Revised version received
25 February 1994
Accepted for publication
19 July 1994

over salbutamol in the treatment of asthma.¹²

Non-selective adrenergic agents, through alpha-mediated vasoconstriction, might be expected to have a different effect on the bronchial and pulmonary vasculature than β agonists. For example, in an animal model of asthma microvascular leakage induced by platelet activating factor was inhibited by adrenaline, whilst the selective β_2 agonist salbutamol had no effect.¹² In acute asthma it has been shown that, whereas salbutamol produces a small fall in PaO_2 , due to bronchial arteriolar dilatation and consequent redistribution of pulmonary blood to poorly ventilated areas, adrenaline produced a small but consistent rise in PaO_2 , suggesting an improvement in the ventilation/perfusion relationship due to vasoconstriction.¹³

The aim of our study was therefore to compare the relative potencies of adrenaline (a non-selective adrenergic agonist) and salbutamol (a β_2 selective 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.

Methods

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_4 1.2, NaH_2PO_4 1.2, CaCl 2.5, NaHCO_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°C continuously gassed with 95% O_2 /5% CO_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, JJ 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 10^{-7} – 10^{-3} 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.¹⁴ Rings were excluded from further study if the initial contraction to 10^{-3} 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 10^{-8} mol/l and the maximum relaxant effect noted. This was followed by serial tenfold increases in cumulative bath concentrations up to 10^{-5} 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 concentration-response was similar for salbutamol and adrenaline. Immediately after the maximum relaxant effect of the last dose was established (10^{-5} mol/l for both agents, a further cumulative histamine concentration-response study was performed in an identical manner. Relaxation and contraction concentration-response curves were each performed over 10–15 minutes.

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.

Analysis

All contractile responses were expressed as a percentage of the initial maximal response to 10^{-3} 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_{20} and EC_{50}) and the mean values before and after the drug were compared using analysis of variance. In addition, the maximum contractile responses to 10^{-3} 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_{20} and EC_{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.

IN VIVO STUDY

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 (FEV_1) $>60\%$ of their predicted value and had previously demonstrated an improvement in FEV_1 of greater than 15% after 200 μg inhaled salbutamol. They also had a provocative dose of histamine causing a 20% fall in FEV_1 ($PD_{20} FEV_1$ hist) of $<4 \mu\text{mol}$. 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

FEV_1 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.*¹⁵ using DeVilbiss No 40 hand-held nebulisers (DeVilbiss Co, Pennsylvania, USA). After baseline FEV_1 had been measured, subjects inhaled 0.9% saline followed by doubling doses (0.03–8 μmol) of histamine with measurement of FEV_1 60 seconds after each inhalation. The challenge was discontinued when the FEV_1 had fallen by 20% or more from post saline values. $PD_{20} FEV_1$ 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 FEV_1 were made, and a histamine challenge test performed. One hour later, provided the FEV_1 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, Sun-

derland, 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 FEV_1 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 $PD_{20} FEV_1$ hist values were log transformed before analysis and expressed as geometric mean values. The change in FEV_1 following adrenaline and salbutamol and the differences in $PD_{20} FEV_1$ hist between adrenaline and salbutamol were compared using Student's paired *t* test. Differences in geometric mean values of $PD_{20} FEV_1$ 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^{-5} mol/l) and (B) adrenaline (10^{-5} mol/l). Values shown are mean (SE) ($n=13$).

Figure 3 Effect of increasing doses of salbutamol and adrenaline on airway calibre in asthmatic subjects in vivo. Values shown are mean (SE) ($n=8$). * $p<0.001$ from baseline values.

Relaxant potency

Figure 1 shows the relaxation curves for both bronchodilators against inherent tone. The mean (SE in log units) EC_{20} and EC_{50} 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 EC_{20} and EC_{50} 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 EC_{20} to histamine, and for adrenaline this was so considerable that the EC_{50} could not be calculated for nine of the 13 preparations. The mean (SE in log units) increase in EC_{20} after salbutamol was 2.4 (0.15) doubling doses. For adrenaline the mean (SE in log units) change in EC_{20} was 5.2 (0.18) doubling doses. The mean difference in the change in EC_{20} 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) FEV_1 rose from 2.96 (0.26) l to 3.41 (0.25) l after the maximum dose of adrenaline (400 µg) and from 2.95 (0.25) l to 3.52 (0.28) l after the maximum dose of salbutamol (400 µg). Figure 3 shows the response of FEV_1 to adrenaline and salbutamol at the different doses. Both drugs produced significant bronchodilation 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) PD_{20} 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 PD_{20} 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 FEV_1 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 used^{18–20} 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 vitro,²⁶ 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 bronchodilation 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.

Figure 4 Histamine PD_{20} before and after 400 µg salbutamol or 400 µg adrenaline in asthmatic subjects in vivo. Values are mean (SE) ($n=8$).

This was the opposite of what we would have expected if the α effects of adrenaline are important. The change we saw with salbutamol is in agreement with several other studies in asthma.^{16 27-30} The only two previous studies to look at the effect of adrenaline on histamine responsiveness *in vivo* looked at physiological concentrations given intravenously,^{31 32} 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 α to β 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 bronchoconstrictor challenges has previously been shown in studies comparing salbutamol and ipratropium.^{16 27}

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 β_2 selective drug, terbutaline.³³ The authors inferred from their study that β_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 β_2 receptor whereas salbutamol is only a partial agonist.³⁴ It is unlikely to represent an α effect as α adrenergic blockade has previously been shown to alter histamine-induced contraction in human airway smooth muscle,²⁴ and although airway smooth muscle from several animal species contains β_1 receptors, it has been difficult to demonstrate them in autoradiographic studies of human airway smooth muscle.³⁵ It might thus be that the partial agonist effect of salbutamol on β_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 β_2 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 α vasoconstrictor effects. The balance of its effects on the vasculature would be vasoconstrictor as it has much more potent α than β_1 effects.

There are three mechanisms whereby vasoactive substances might alter bronchial responsiveness, apart from a direct effect 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.² The lack of effect of adrenaline *in vivo* would suggest that a reduction in the vascular clearance of histamine due to the vasoconstrictor properties of adrenaline may have negated the other potentially beneficial effects on the vasculature and airway smooth muscle.

Further evidence to support this hypothesis is that reduction of tracheobronchial blood flow in dogs prolongs methacholine-induced bronchial obstruction,¹⁰ and in man methacholine-induced bronchial obstruction is prevented by a potent vasodilator, prostacyclin.¹¹ Similarly, allergen-induced bronchoconstriction in sensitised sheep is prolonged by the vasoconstrictor agent vasopressin, and reduced by the vasodilator nitroglycerin.⁹

The lack of effect of adrenaline on histamine responsiveness in our study contrasts with two studies showing that the α agonists methoxamine and noradrenaline protect against exercise and hyperventilation-induced airflow obstruction respectively.^{7 8} 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,³ 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.³⁶

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*¹⁹ found no significant difference between salbutamol and adrenaline in bronchodilatation over a 60 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 α_2 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 β_2 effects, possibly by reducing vascular clearance of histamine.

The authors thank Mr Morgan, Mr Salama and Professor Jones at Nottingham City Hospital for providing human lung tissue, and Hilary Hughes for secretarial support.

- Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013–26.
- Lockhart A, Dinh-Xuan AT, Regnard J, Cabanes L, Matran R. Effect of airway blood flow on airflow. *Am Rev Respir Dis* 1992;146:S19–23.
- McFadden ER Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon. *Lancet* 1990;335:880–3.
- Widdicombe J. Physiologic control. Anatomy and physiology of the airway circulation. *Am Rev Respir Dis* 1992;146:S3–7.
- Freedman RJ. The functional geometry of the bronchi. The relationship between changes in external diameter and calibre, and a consideration of the passive role played by the mucosa in bronchoconstriction. *Bull Eur Physiopathol Respir* 1972;8:45–52.
- Moreno R, Hogg JC, Pare PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986;133:1171–80.
- Dinh-Xuan AT, Chausain M, Regnard J, Lockhart A. Pretreatment with an inhaled α_1 -adrenergic agonist methoxamine reduces exercise-induced asthma. *Eur Respir J* 1989;2:408–14.
- Gilbert IA, McFadden ER Jr. Airway cooling and re-warming: the second reaction sequence in exercise-induced asthma. *J Clin Invest* 1992;90:699–704.
- Csete ME, Chediak AD, Abraham WM, Wanner A. Airway blood flow modifies allergic airway smooth muscle contraction. *Am Rev Respir Dis* 1991;144:59–63.
- Wagner EM, Mitzner WA. Bronchial circulatory reversal of methacholine induced airway constriction. *J Appl Physiol* 1990;69:1220–4.
- Hardy CC, Bradding P, Robinson C, Holgate ST. Bronchoconstrictor and antibronchoconstrictor properties of inhaled prostacycline in asthma. *J Appl Physiol* 1988;64:1564–7.
- Boschetto P, Roberts NM, Rogers DF, Barnes PJ. Effect of anti-asthma drugs on microvascular leakage in guinea-pig airways. *Am Rev Respir Dis* 1989;139:416–21.
- Coupe MO, Guly U, Brown E, Barnes PJ. Nebulised adrenaline in acute severe asthma; comparison with salbutamol. *Eur J Respir Dis* 1987;71:227–32.
- Knox AJ, Ajao P, Britton JR, Tattersfield AE. Effect of sodium transport inhibitors on airway smooth muscle contractility in vitro. *Clin Sci* 1990;79:315–23.
- Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760–5.
- Higgins BG, Powell RM, Cooper S, Tattersfield AE. Effect of salbutamol and ipratropium bromide on airway calibre and bronchial reactivity in asthma and chronic bronchitis. *Eur Respir J* 1991;4:415–20.
- Phillips GD, Finnerty JP, Holgate ST. Comparative protective effect of the inhaled β_2 agonist salbutamol (albuterol) on bronchoconstriction provoked by histamine, methacholine and adenosine 5'-monophosphate in asthma. *J Allergy Clin Immunol* 1990;85:755–62.
- Wong CS, Pavord ID, Williams J, Britton JR, Tattersfield AE. Bronchodilator, cardiovascular, and hypokalaemic effects of fenoterol, salbutamol, and terbutaline in asthma. *Lancet* 1990;336:1396–9.
- Kjellman B, Tolig H, Wettrell G. Inhalation of racemic epinephrine in children with asthma. *Allergy* 1980;35:605–10.
- Phanichyakarn P. Comparison of subcutaneous injections of terbutaline, salbutamol and adrenaline in acute asthmatic attacks in children. *J Med Assoc Thai* 1989;72:692–6.
- Giner MT, Nevot S, Sierra JL, Plaza A, Coma G, Youssef W. Treatment of acute crises of bronchial asthma in children using subcutaneous salbutamol as an alternative to adrenaline; comparative study. *An Esp Pediatr* 1986;25:165–9.
- Advenier CE, Naline E, Matran R, Toty L, Bakdach H. Interaction between fenoterol, ipratropium, and acetylcholine on human isolated bronchus. *J Allergy Clin Immunol* 1988;82:40–6.
- Goldie RG, Paterson JW, Spina D, Wale JL. Classification of β -adrenoceptors in human isolated bronchus. *Br J Pharmacol* 1984;81:611–5.
- Mathe AA, Astrom A, Perssch NA. Some bronchoconstricting and bronchodilating responses of human isolated bronchi: evidence for the existence of α -adrenoceptors. *J Pharm Pharmacol* 1971;23:905–10.
- Zaagsma J, Van der Heijden PJCM, Van der Schaaf MWG, Bank CMC. Comparison of functional β -adrenoceptor heterogeneity in central and peripheral airway smooth muscle on guinea pig and man. *J Receptor Research* 1983;3:89–106.
- Olsson AOT, Swanberg E, Svedinger I, Waldeck B. Effect of β -adrenoceptor agonists on airway smooth muscle and on slow-contracting skeletal muscle: in vitro and in vivo results compared. *Acta Pharmacol Toxicol* 1979;44:272–6.
- Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax* 1988;43:300–5.
- Casterline CL, Evans R, Ward GW. The effect of atropine and albuterol aerosols in the human bronchial response to histamine. *J Allergy Clin Immunol* 1976;58:607–13.
- Chung KF, Morgan B, Keyes SJ, Snashall PD. Histamine dose response relationship in normal and asthmatic subjects. *Am Rev Respir Dis* 1982;126:849–54.
- Cockcroft DW, Killian N, Mellon JA, Hargreave FE. Protective effect of drugs on histamine induced asthma. *Thorax* 1977;32:429–37.
- Knox AJ, Campos-Gongora H, MacDonald IA, Tattersfield AE. Modification of bronchial reactivity by physiological concentrations of plasma epinephrine. *J Appl Physiol* 1992;73:1004–7.
- Warren JB, Dalton N, Turner C, Clark TJH. Protective effect of circulating epinephrine within the physiological range on the airway response to inhaled histamine in non-asthmatic subjects. *J Allergy Clin Immunol* 1984;74:683–6.
- Gustafsson B, Persson CGA. Effect of different bronchodilators on airway smooth muscle responsiveness to contractile agents. *Thorax* 1991;46:360–5.
- Tattersfield AE, Britton JR. β -adrenoceptor agonists. In: *Asthma: basic mechanisms and clinical management*. 2nd edn. London: Academic Press, 1992:527–54.
- Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualisation of β -adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985;132:541–7.
- Gilbert IA, Winslow CJ, Lenner KA, Nelson JA, McFadden ER Jr. Vascular volume expansion and thermally induced asthma. *Eur Respir J* 1993;6:189–97.
- Gründström N, Andersson RGG, Witberg JES. Pre-junctional α_1 adrenoceptors inhibit contraction of tracheal smooth muscle by inhibiting cholinergic neurotransmission. *Life Sci* 1981;28:2981–6.