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Pharmacological modulation of bronchial anaphylaxis induced by aerosol challenge in anaesthetized guinea-pigs

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1 Anaphylactic bronchoconstriction provoked by aerosol challenge, and its pharmacological modulation, has been investigated in anaesthetized pump-ventilated guinea-pigs actively sensitized to ovalbumin (OA).

2 Aerosol challenge (OA 0.03–10 mg ml^{-1}) provoked immediate bronchoconstriction, the degree of which, and its rate of development, was directly related to antigen concentration.

3 Concomitant changes in heart rate and systemic arterial blood pressure following aerosol challenge were reduced compared with systemic (OA, 1 mg kg^{-1} i.v.) challenge. Unlike systemic challenge, aerosol challenge did not cause a concomitant fall in either circulating leukocyte or platelet count.

4 When a submaximal (microshock) aerosol challenge stimulus (OA, 0.3 mg ml^{-1}, 5 s) was employed, bronchoconstriction was markedly reduced by mepyramine (2 mg kg^{-1} i.v.). The residual component of bronchoconstriction was enhanced by indomethacin (10 mg kg^{-1} i.v.), an effect which was reversed by either BW755C (30 mg kg^{-1} i.v.) or FPL55712 (10 mg kg^{-1} i.v.).

5 When a supramaximal (macroshock) aerosol challenge stimulus (OA, 10 mg ml^{-1}, 5 s) was employed, bronchoconstriction was also markedly reduced by mepyramine. Residual bronchoconstriction was not altered by indomethacin, slowed but not reduced by indomethacin plus BW755C, and substantially reduced by indomethacin plus FPL55712.

6 The successive incremental antagonism of anaphylactic bronchoconstriction by mepyramine and mepyramine plus indomethacin and FPL55712 indicates that the predominant mediators involved are histamine and leukotrienes, respectively. The failure of indomethacin plus BW755C to inhibit the mepyramine-resistant bronchoconstriction provoked by OA macroshock may reflect the increased generation of leukotrienes via a 5-lipoxygenase isoenzyme resistant to inhibition by BW755C.

7 Aerosol challenge of actively sensitized anaesthetized guinea-pigs by this method may be a useful model of human allergic bronchoconstriction, particularly when the effects of a drug given itself as an aerosol are being evaluated.

Introduction

Despite long-recognised immunopharmacological differences from human asthma, anaphylactic bronchoconstriction in guinea-pigs remains a popular laboratory model of this disease (Kallos & Kallos, 1984). This is mainly because of the relative ease with which immediate allergic bronchoconstriction can be provoked in this species, and the demonstrable role of other humoral factors (Collier & James, 1967), notably prostanoids and leukotrienes, in this process. These mediators, in particular leukotrienes, have been implicated as important spasmogens in human asthma and may also contribute to the development of airway hyperreactivity (Lewis, 1985; Robinson & Holgate, 1985). Indeed, recent studies have described the pharmacological manipulation of anaphylactic bronchospasm in guinea-pigs to isolate and exaggerate its leukotriene-dependent component (Ritchie *et al.*, 1981; Anderson *et al.*, 1983). However, in these studies, and in many earlier studies, the route of antigen challenge was by intravenous administration giving rise to a primarily systemic, rather than pulmonary, allergic response.

A more obvious analogy with human asthma is where the route of antigen challenge is by inhalation.

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To this end we have recently described a simple method of inducing anaphylactic bronchospasm in actively sensitized anaesthetized guinea-pigs by direct inhalation of antigen in an aerosol form (Payne & Nucci, 1987). In the present study we have characterized further our *in vivo* method of aerosol challenge. Moreover, using this method, we have also investigated the pharmacological modulation of allergic bronchospasm induced by either sub- or supra-maximal antigen challenge. This has enabled us to identify and compare the respective contribution of different anaphylactic mediators in these two situations.

Methods

Sensitization procedure

Male Dunkin Hartley guinea-pigs (250–300 g) were actively sensitized to ovalbumin (Grade V; Sigma) by intraperitoneal injection of 50 mg of this protein, together with a further 50 mg given subcutaneously (each in 1 ml of 0.9% saline).

Antigen challenge

Fourteen to twenty one days after sensitization animals selected randomly were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and placed in a dorsal recumbent position on a small animal operating table. The trachea was cannulated and the lungs ventilated mechanically by a Searle BioScience constant volume respiration pump (54 strokes min^{-1} of 1 ml laboratory air per 100 g body wt). Both cervical vagi nerves were transected at the level of the neck. Pulmonary inflation pressure (PIP) was measured from a lateral port in the afferent limb of the ventilator circuit, with a pressure transducer (Elcomatic EM750 or Statham P23ID). Systemic arterial blood pressure (BP) heart rate were recorded continuously from a catheter placed in the left carotid artery and connected to a pressure transducer (Elcomatic EM759 or Statham Pb21AA) and a cardiotachometer coupler (Beckman 9857B or Grass 7P44B) on either a Beckman type R611 dynograph, or a Grass model 7D polygraph. Typical resting values were PIP 10–15 cmH_2O; BP 50/40 mmHg (systolic/diastolic); heart rate 270 beats min^{-1}. Rectal temperature was maintained at 37°C by heat from a thermostatically controlled infra-red lamp. A jugular vein was also cannulated for intravenous administration of drugs.

After a 10 min stabilization period, or after drug pretreatment each animal was challenged with the sensitizing antigen by direct inhalation. This was achieved by administering an aerosol of ovalbumin generated by a DeVilbiss 'PulmoSonic' Ultrasonic nebulizer permanently connected in series with the afferent limb of the ventilator circuit (Payne & Nucci, 1987). The aerodynamic diameter of the particles generated by the nebulizer under these conditions ranged from 0.6 to 15 μm with a modal particle size of 1.3 μm as measured by a TSI particle sizer (M.J.S. Gazeley, Wellcome, personal communication). For the purpose of comparison, in some experiments animals were challenged intravenously with a bolus injection of ovalbumin (1 mg kg^{-1}).

Haematological profiling

In some animals arterial blood samples (1 ml) were collected from the carotid catheter into tri-sodium citrate buffer (final concentration 0.32%) 10 min before and 10 min after antigen challenge. The haematocrit, red cell and leukocyte count in each sample were measured using a Clay Adams HA5 haematology analizer. The number of circulating platelets was measured using a Clay Adams Ultra-Flo 100 whole blood platelet counter.

Drugs

The following drugs were used: sodium pentobarbitone (Sagatal), mepyramine maleate (BDH), indomethacin (Sigma), BW755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) (Wellcome) and FPL55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) (a gift from Dr. P. Sheard, Fisons PLC, Loughborough).

Sodium pentobarbitone was supplied as a solution of 60 mg ml^{-1} which was diluted to 10 mg ml^{-1} with 0.9% w/v NaCl solution (saline). Fresh stock solutions of the other drugs were prepared daily as follows: mepyramine maleate 2 mg ml^{-1} in saline; BW755C and FPL55712 10 mg ml^{-1} each in double distilled deionized H_2O; indomethacin 10 mg ml^{-1} in 1 M Tris buffer pH 8.5.

Statistical analysis

Results are presented as mean ± s.e.mean. Differences between means were analysed by Students *t* test for unpaired data. A probability level of $P < 0.05$ was considered statistically significant.

Results

Antigen concentration

Our first objective was to investigate the effect of challenge, by inhalation, with aerosols of OA generated from stock solutions of different concentrations (0.03–10 mg ml^{-1}) of this antigen in saline. The time of

administration of the aerosol was kept constant at 5 s, and the volume of OA solution in the aerosol reservoir was always 10 ml. In control experiments challenge of either non-sensitized animals with OA aerosol, or of sensitized animals with saline aerosol, was without effect. However challenge of sensitized animals with OA aerosol provoked acute bronchoconstriction (rise in PIP), the degree of which was directly related to the concentration of the solution of antigen from which the aerosol was generated (Figure 1). The maximal rise in PIP obtained ($57 \pm 4.0\,cmH_2O$; $n = 5$) with the highest antigen concentration (OA $10\,mg\,ml^{-1}$) represented approximately 85% of the maximum theoretical increase possible (i.e. with the tracheal catheter manually totally occluded). In all cases there was an initial lag phase of between 15 to 60 s after challenge before a rise in PIP was evident. Both this lag phase, and the rate at which bronchoconstriction developed thereafter were directly-related to antigen concentration (Figure 2). Figure 2 also illustrates the sustained nature of anaphylactic bronchospasm in the 10 min following aerosol challenge; in almost all instances PIP did not return to pre-challenge values even at 1 h post-challenge (data not shown).

Cardiovascular effects

As well as provoking anaphylactic bronchoconstriction, aerosol challenge of sensitized (but not non-sensitized) animals also caused concomitant alterations in both mean systemic arterial blood pressure (MABP) and heart rate (HR). The size and direction of these changes were dependent on the concentration of

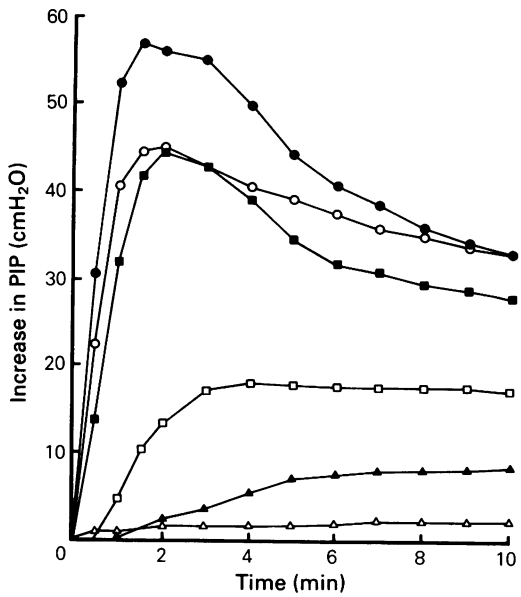

Figure 2 The time course of the rise in pulmonary inflation pressure (PIP) provoked by 5 s challenge of actively-sensitized anaesthetized guinea-pigs with increasing concentrations of ovalbumin (OA) aerosol: (△), $0.03\,mg\,ml^{-1}$; (▲), $0.1\,mg\,ml^{-1}$; (□), $0.3\,mg\,ml^{-1}$; (■), $1\,mg\,ml^{-1}$; (○), $3\,mg\,ml^{-1}$; (●), $10\,mg\,ml^{-1}$. Each point represents the mean result from individual groups of 5 animals. S.e.means have been omitted for clarity but for any single point did not exceed $5.0\,cmH_2O$.

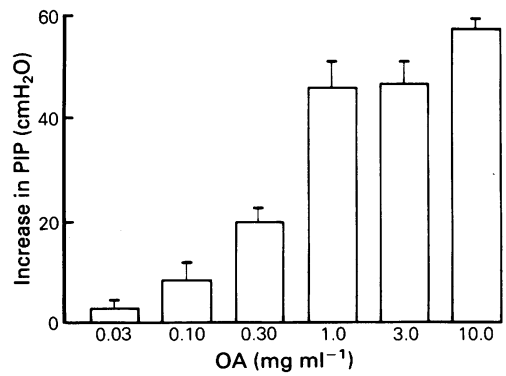

Figure 1 Peak instantaneous rise in pulmonary inflation pressure (PIP) measured in the first 10 min following a 5 s challenge of actively-sensitized anaesthetized guinea-pigs with inhaled aerosols generated from increasing concentrations of ovalbumin (OA) solution. Each column represents the mean result from individual groups of 5 animals; vertical lines indicate s.e.mean.

sustained maximum fall in MABP of 8.7 ± 2.3 mmHg ($n = 5$; $P < 0.05$), coupled with a maximum drop in HR of 18.0 ± 6.0 beats min^{-1} ($P < 0.05$). Aerosol challenge with higher antigen concentrations ($1-10\,mg\,ml^{-1}$) also provoked an initial fall in MABP (in the range of 10 mmHg). However, this was transient and was rapidly followed by a sustained net rise in MABP of up to 26 ± 6.0 mmHg with a simultaneous rise in HR up to a maximum of 29 ± 9 beats min^{-1} ($n = 5$). It is of interest to note that whereas i.v. challenge (OA $1\,mg\,kg^{-1}$) provoked a similar maximal increase in PIP (up to $67.1 \pm 3.1\,cmH_2O$) to aerosol challenge, the accompanying cardiovascular effects were exaggerated (rise in MABP of 58 ± 5 mmHg and rise in HR of 72 ± 14 beats min^{-1}; $n = 5$).

Haematology

Haematological analysis of arterial blood samples revealed no statistically significant change in either leukocyte or platelet count following aerosol challenge (OA; $10\,mg\,ml^{-1}$, 5 s). In contrast, following i.v.

antigen and thus the magnitude of the provocation stimuli. At low antigen concentrations (0.03–0.3 mg ml^{-1}) aerosol challenge provoked a small but challenge (OA; 1 mg kg^{-1}) there was a marked reduction of 70–80% in both the number of circulating leukocytes and platelets (Figure 3). Red blood cell count and haematocrit were not significantly altered following challenge by either route (data not shown).

Pharmacological modulation

To investigate the nature and respective contributions of the anaphylactic mediators involved following aerosol challenge, two challenge stimuli were employed. These were aerosols generated from stock solutions of OA at either 0.3 mg ml^{-1} or 10 mg ml^{-1}, in each case administered for 5 s, which have been termed 'OA microshock' and 'OA macroshock', respectively. In this study the following antagonists/inhibitors were employed, either alone or in combination: the histamine H_1-receptor antagonist mepyramine the SRS-A antagonist FPL55712 the cyclo-oxygenase inhibitor indomethacin and the dual cyclo-oxygenase/lipoxygenase inhibitor BW755C.

Mepyramine and indomethacin (either alone or in combination) were given 10 min before antigen challenge, BW755C 5 min before, and FPL55712 1 min before challenge, respectively. The dose volume was always 1 ml kg^{-1} i.v., except in the case of BW755C where it was 3 ml kg^{-1} i.v.

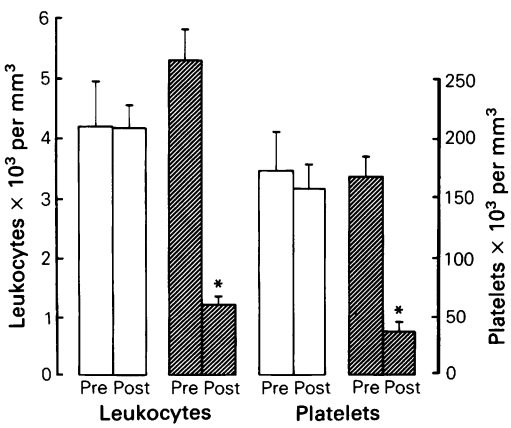

Figure 3 Comparison of the effect of aerosol (ovalbumin (OA), 10 mg ml^{-1}, 5 s; open columns) and intravenous (OA, 1 mg kg^{-1}; hatched columns) antigen challenge on circulating leukocytes and platelets in actively-sensitized anaesthetized guinea-pigs. In each case pre (−10 min) and post (+10 min) challenge values are shown, each column representing the mean result from individual groups of 3 animals; vertical lines indicate s.e.mean * $P<0.05$ vs pre-challenge values.

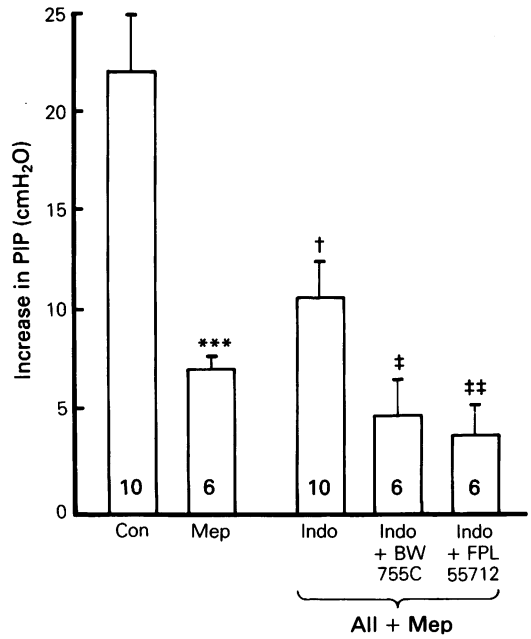

Figure 4 Effect of pretreatment with various combinations of inhibitors and antagonists intravenously (see text for details) on the peak instantaneous rise in pulmonary inflation pressure (PIP) measured over the first 10 min following a submaximal (microshock) aerosol challenge stimulus (ovalbumin (OA), 0.3 mg ml^{-1}, 5 s) in actively-sensitized anaesthetized guinea-pigs. All columns represent the mean result from individual groups of n animals (as indicated at the foot of each column); vertical lines indicate s.e.mean Con = control, Mep = mepyramine (2 mg kg^{-1} i.v.), Indo = indomethacin (10 mg kg^{-1} i.v.). BW755C was used at a dose of 30 mg kg^{-1} i.v. and FPL55712 at 10 mg kg^{-1} i.v. ***$P<0.005$ vs control; †$P<0.05$ vs mepyramine; ‡$P<0.05$ vs mepyramine + indomethacin ‡‡$P<0.01$ vs mepyramine + indomethacin.

Ovalbumin microshock

The effect of various combinations of the above inhibitors/antagonists on the magnitude of anaphylactic bronchoconstriction provoked by OA microshock (OA; 0.3 mg ml^{-1}, 5 s); and its time course, is shown in Figures 4 and 5, respectively. In control animals, the peak rise in PIP provoked by OA microshock was 22.3 ± 2.8 cmH_2O (n = 10). Pretreatment with mepyramine (2 mg kg^{-1} i.v.) reduced this to 7.3 ± 1.9 cmH_2O (n = 6; $P<0.005$). Mepyramine also slowed both the onset and development of bronchospasm. After additional pretreatment with indomethacin (10 mg kg^{-1} i.v.), the maximum rise in PIP attained 10 min after aerosol challenge was elevated to

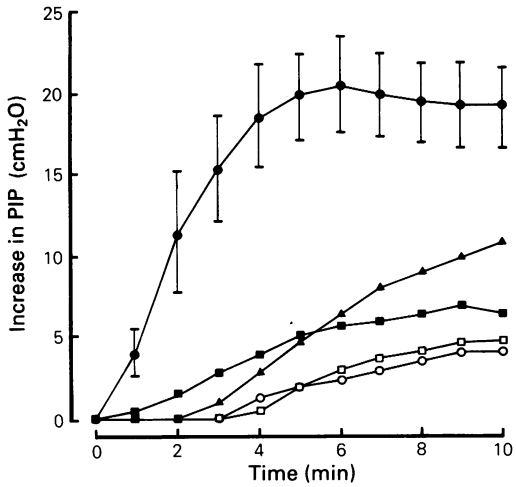

Figure 5 Effects of pretreatment with various combinations of inhibitors/antagonist intravenously (see text for details) on the time course of the rise in pulmonary inflation pressure (PIP) provoked by a submaximal (microshock) aerosol challenge stimulus (ovalbumin, 0.3 mg ml^{-1}, 5 s) in acively-sensitized anaesthetized guinea-pigs; (●) control; (■) mepyramine, 2 mg kg^{-1}; (▲) mepyramine and indomethacin, 10 mg kg^{-1}; (□) mepyramine, indomethacin and BW755C, 30 mg kg^{-1}; (○) mepyramine, indomethacin and FPL55712, 10 mg kg^{-1}. Each point represents the mean result from individual groups of 6–10 animals; vertical lines (control) indicate s.e.means.

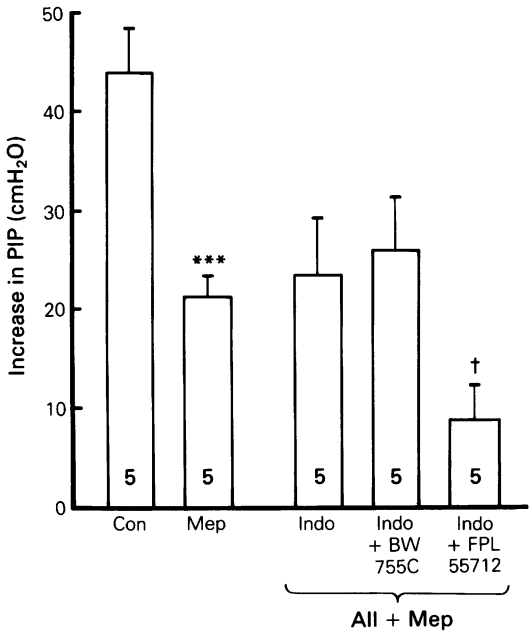

Figure 6 Effect of pretreatment with various combinations of inhibitors and antagonists intravenously (see text for details) on the peak instantaneous rise in pulmonary inflation pressure (PIP) measured over the first 10 min following a supramaximal (macroshock) aerosol challenge stimulus (ovalbumin, 10.0 mg ml^{-1}, 5 s) in actively-sensitized anaesthetized guinea-pigs. All columns represent the mean result from individual groups of n animals (as indicated at the foot of each column); vertical lines indicate s.e.mean Con = control, Mep = mepyramine (2 mg kg^{-1} i.v.), Indo = indomethacin (10 mg kg^{-1} i.v.). BW755C was used at a dose of 30 mg kg^{-1} i.v. and FPL55712 at 10 mg kg^{-1} i.v. ***$P < 0.005$ vs control; $^{\dagger}P < 0.05$ vs mepyramine + indomethacin.

10.8 ± 1.5 cmH_2O ($n = 10$). This small, but statistically significant ($P < 0.05$), enhancement by indomethacin of the mepyramine-resistant reduction in airway calibre was reversed by further pretreatment with either BW755C (30 mg kg^{-1} i.v.) or FPL55712 (10 mg kg^{-1} i.v.), the maximum rise in PIP being 4.1 ± 1.5 and 4.8 ± 2.0 cmH_2O, respectively ($P < 0.05$ in each case, $n = 6$,). As well as this approximate 60% reduction in the rise in PIP compared to animals pretreated with mepyramine and indomethacin alone, both BW755C and FPL55712 further slowed both its onset and overall time course (see Figure 5).

In the absence of mepyramine pretreatment, indomethacin (10 mg kg^{-1}) was without effect on either the magnitude or time course of OA microshock (data not shown).

Ovalbumin macroshock

The effects of the same inhibitor/antagonist combinations as described above were also investigated using a supramaximal aerosol challenge stimulus, OA macroshock (OA; 10 mg ml^{-1}, 5 s) and are shown in Figures 6 and 7. Mepyramine (2 mg kg^{-1} i.v.) markedly reduced the peak rise in PIP following aerosol challenge from 44 ± 4 cmH_2O ($n = 6$) in control animals, to 21.5 ± 2 cmH_2O ($n = 6$) and abolished the secondary cardiovascular pressor response. The onset and subsequent development of the rise in PIP was also slowed. In contrast to the mepyramine-resistant rise in PIP induced by OA microshock, that induced by OA macroshock was not significantly modified (either in magnitude or time course) by additional pretreatment with indomethacin (10 mg kg^{-1} i.v.). Similarly, further pretreatment with BW755C (30 mg kg^{-1} i.v.) had no statistically significant effect on the peak rise in PIP provoked by OA macroshock. However, BW755C did slow the onset of anaphylactic bronchospasm under these pretreatment conditions to 100.8 ± 7.9 s as opposed to 68.4 ± 10.7 s ($P < 0.05$) in animals receiv-

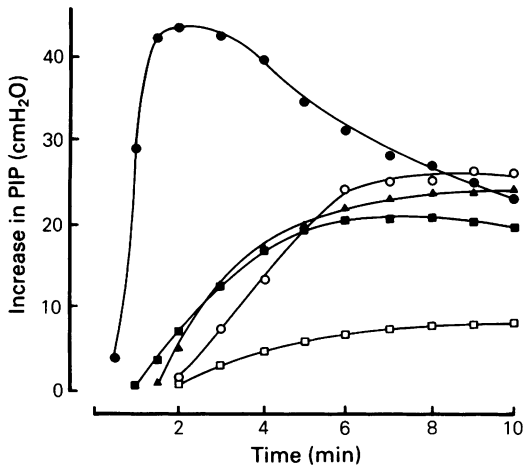

Figure 7 Effect of pretreatment with various combinations of inhibitors/antagonists intravenously (see text for details) on the time course of the rise in pulmonary inflation pressure (PIP) provoked by a supramaximal (microshock) aerosol challenge stimulus (ovalbumin, 10.0 mg ml^{-1}, 5 s) in actively-sensitized anaesthetized guinea-pigs: (●) control; (■) mepyramine, 2 mg kg^{-1}; (▲) mepyramine and indomethacin, 10 mg kg^{-1}; (○) mepyramine, indomethacin and BW755C, 30 mg kg^{-1}; (□) mepyramine, indomethacin and FPL55712, 10 mg kg^{-1}. Each point represents the mean result from individual groups of 6–10 animals. S.e.means have been omitted for clarity, but for any single point did not exceed 6.0 cmH_2O.

ing mepyramine plus indomethacin alone. Whereas BW755C did not modify peak bronchospasm in this situation, FPL55712 (10 mg kg^{-1} i.v.) markedly attenuated the rise in PIP provoked by OA macroshock following indomethacin plus mepyramine. Thus the maximum rise in PIP following FPL55712 was 8.0 ± 4.0 cmH_2O ($P < 0.05$), a reduction of approximately 65% as compared to the change in PIP in animals pretreated with mepyramine plus indomethacin alone. In the absence of mepyramine pretreatment, at the above doses neither indomethacin nor BW755C alone altered either the magnitude or time course of OA macroshock (results not shown).

Discussion

We have previously described a method for provoking anaphylactic bronchospasm in actively-sensitized anaesthetized guinea-pigs by direct inhalation of antigen in an aerosol form (Payne & Nucci, 1987). This technique allows simultaneous quantitative measurement of the consequent reduction in airway calibre by determining changes in pulmonary inflation pressure. We believe that this method of challenge is preferable to the intravenous route, since the antigen is delivered directly to the airway smooth muscle rather than via the vasculature. Furthermore, both the physiological and pathological sequelae of aerosol challenge in guinea-pigs are known to closely resemble human asthma (Kallos & Kallos, 1984).

The present study extends the knowledge of the nature and respective contribution of the anaphylactic mediators involved in the response of actively-sensitized guinea-pigs to antigen in an aerosol form. We have shown that by varying the antigen concentration, a graded level of anaphylactic bronchospasm can be obtained. At the highest antigen challenge concentration, there was no evidence of either activation of blood elements or pulmonary entrapment of platelets. This is clearly shown by the unchanged numbers of circulating leukocytes and platelets. In contrast there was a very marked drop in both these cell types following i.v. challenge, confirming previous findings by other workers (Darius *et al.*, 1986; Pretolani *et al.*, 1986). Intravenous challenge was also accompanied by substantial cardiovascular changes in comparison to aerosol challenge. These results support our contention that in contrast to i.v. challenge, aerosol challenge induces a more selective bronchial reaction, with reduced systemic effects.

In the guinea-pig, anaphylactic bronchospasm following i.v. challenge is markedly attenuated and slowed by pretreatment with a histamine H_1-receptor antagonist (Ritchie *et al.*, 1981; Anderson *et al.*, 1983). Similarly in the present study, mepyramine strongly antagonized the peak rise in PIP following aerosol challenge. Mepyramine also slowed the onset of bronchospasm. We conclude therefore that the predominant early bronchoactive mediator released following aerosol challenge is also histamine, followed by the later release of other spasmogens. In this respect, and in contrast to Lewis *et al.* (1983), we found no evidence of a greater proportion of histamine H_1-receptor antagonist-insensitive bronchospasm with increasing provocation stimuli. Instead, our results point to an overall increase in anaphylactic mediator-release rather than a shift in the balance of the histamine-dependent and histamine-independent components.

The delay in onset of mepyramine-resistant anaphylactic bronchospasm is entirely consistent with the synthesis and release of other mediators such as leukotrienes. It is similar to the delay seen in both the Schultz-Dale reaction in isolated tissues (Forsberg & Sorenby, 1979) and following i.v. challenge in the presence of a histamine H_1-receptor antagonist (Ritchie *et al.*, 1981; Anderson *et al.*, 1983). In the latter situation, indomethacin had been used to enhance histamine-independent resistant bronchospasm. This

is arguably through directing the immunologically activated metabolism of arachidonic acid through the lipoxygenese pathway (increased generation of leukotrienes) rather than the cyclo-oxygenase pathway. Although this concept has been recently questioned (Burka, 1985) such 'redirection' may be more prominent when antigen challenge is via the airways (Nucci *et al.*, 1986). We found indomethacin to be without effect on anaphylactic bronchospasm provoked by either micro- or macro-shock in the absence of mepyramine pretreatment. Furthermore, following mepyramine pretreatment, indomethacin did not enhance anaphylactic bronchospasm provoked by a macroshock challenge stimulus, nor did it influence its time course. However, indomethacin did cause a small enhancement of mepyramine-resistant bronchospasm provoked by a low (microshock) challenge stimulus. Following indomethacin both the enhanced bronchospasm provoked by OA microshock and the unmodified bronchospasm provoked by OA macroshock were equally inhibited by the putative SRS-A antagonist FPL55712. Thus in both cases the predominant mediators responsible for the residual bronchospasm in the presence of anti-histamine and cyclo-oxygenase blockade appear to be leukotrienes, as has been previously found with i.v. challenge (Ritchie *et al.*, 1981; Anderson *et al.*, 1983). It is possible that following a low challenge stimulus (microshock), a redirection of arachidonic acid metabolism (with increased leukotriene production) may occur, whereas following a high challenge stimulus (macroshock) and lipoxygenase pathway is already maximally stimulated. The small and slowly developing FPL55712-resistant bronchospasm provoked by aerosol challenge may reflect the spasmogenic action of other lipid-derived mediators such as platelet-activating factor (Paf; Darius *et al.*, 1986; Fitzgerald *et al.*, 1986).

To evaluate further the role of lipoxygenase products in anaphylactic bronchospasm following aerosol challenge we used the dual cyclo-oxygenase/lipoxygenase inhibitor BW755C. This compound has previously been demonstrated to inhibit 'leukotriene dependent' anaphylactic bronchospasm in guinea-pigs following either i.v. (Ritchie *et al.*, 1981; Anderson *et al.*, 1983) or (in passively sensitized animals) aerosol challenge (Saga *et al.*, 1984). In the present study we evaluated the effect of BW755C under the same pretreatment conditions (combination of a histamine H_1-receptor antagonist and indomethacin). However, whilst BW755C inhibited 'leukotriene-dependent' bronchospasm provoked by OA microschock, it was ineffective when a macroshock provocation stimulus was used, although there was evidence that in the latter situation the development of bronchospasm was slowed. Interestingly a similar differential degree of inhibition of anaphylactic bronchospasm by BW755C, depending on the size of the provocation stimuli, has also been observed in rats (Dahlback & Sorenby, 1982). Furthermore, Nucci *et al.* (1986) have recently suggested that there might be two functional 5-lipoxygenase enzymes in guinea-pig lung, one of which may be insensitive to inhibition of BW755C. It is possible therefore that aerosol challenge with a macroshock stimuli *in vivo* might activate a significant and perhaps predominant proportion of leukotriene synthesis via this BW755C resistant 5-lipoxygenase isoenzyme.

In conclusion, direct antigen challenge, by inhalation, of actively-sensitized anaesthetized guinea-pigs, as we have described, may be a useful and potentially more relevant experimental model of human allergic asthma. As the anaphylactic reaction following aerosol challenge is primarily pulmonary rather than systemic, this model may be of particular value when the effects of a drug given itself as an aerosol are being evaluated.

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