British Journal of Pharmacology (1996) 118, 989-997

Effects of local and systemic budesonide on allergen-induced airway reactions in the pig

[1]Cecilia Fornhem, *Magnus Dahlbäck, †Maria Kumlin, Jan M. Lundberg & Kjell Alving

Department of Physiology and Pharmacology, † Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm and *Preclinical R & D, Pharmacology, Astra Draco AB, Lund, Sweden

1 In this study, an attempt was made to distinguish between local and systemic effects of low doses of the topical glucocorticoid, budesonide. The effect of aerosolized budesonide administered to the lower airways versus intravenously administered budesonide on the acute and late response to nebulized *Ascaris suum* extract in the lung, was evaluated in the minipig after active sensitization with purified *A. suum* antigen. Budesonide was administered once, 1 h prior to *A. suum* challenge and airway reactions and mediator release were observed for 8 h after allergen challenge.

2 In the budesonide aerosol group ($n=6$), 10.2 ± 1.2 $\mu g\ kg^{-1}$ budesonide was given locally and in the budesonide infusion group ($n=5$), 5 $\mu g\ kg^{-1}$ was given intravenously. The area under the plasma concentration curve for budesonide during the experiment was 11.4 ± 1.2 and 10.3 ± 1.2 nM h in the budesonide aerosol and budesonide infusion group, respectively (no significant difference). The lung tissue content of budesonide in the two groups was 45.2 ± 4.9 and 18.4 ± 3.5 nmol kg^{-1} dry tissue, respectively, 8 h after allergen challenge ($P<0.05$). For comparison, 6 pigs were given budesonide vehicle as an infusion prior to *A. suum* challenge.

3 Total lung resistance (R_L) increased acutely (maximal response within 15 min) in the budesonide aerosol, budesonide infusion and budesonide vehicle groups (by 91 ± 40, 150 ± 86 and $80\pm27\%$, respectively). The acute reaction partially resolved at about 1 h and was followed by a late increase in R_L in the budesonide infusion and budesonide vehicle groups (by 251 ± 148 and $281\pm136\%$ at 8 h, respectively). However, no late change in R_L was seen in the budesonide aerosol group ($7\pm24\%$).

4 Aerosolized budesonide had a protective effect in that it attenuated the late changes in arterial blood gas and pH as well as the late elevation of plasma catecholamines. Budesonide given as an infusion did not protect against the late changes in these parameters. However, budesonide aerosol or infusion did not inhibit the late vasodilatation in the bronchial circulation.

5 Histamine and cysteinyl-leukotrienes were released during the acute reaction as measured by urinary concentration of methylhistamine and leukotriene E_4 respectively. There was no release of histamine during the late reaction. A late increase in leukotriene E_4 was observed in 2 of the budesonide infusion and 3 of the budesonide vehicle pigs, whereas no such increase was seen in any of the budesonide aerosol pigs.

6 Budesonide concentration in lung tissue, but not in plasma at 8 h correlated negatively with the late increase in R_L ($P<0.05$, $r=-0.53$, $n=10$), whereas budesonide concentration in plasma but not in lung tissue correlated negatively with the late decrease in dynamic compliance ($P<0.05$, $r=-0.67$, $n=12$).

7 This study has shown that a single low dose of locally administered budesonide can inhibit the late allergic reaction in the pig lower airways. If budesonide was given as an intravenous infusion in a dose yielding a plasma concentration similar to that seen after the aerosol treatment, the protective effect of budesonide was poor. It may be suggested that the tissue-bound portion of budesonide affects local mechanisms involved in the development of late changes in the airways (R_L), although it does not affect the late increase in bronchial blood flow. We conclude that the inhibitory effect of budesonide on the allergen-induced late reaction in the pig airways relates to tissue-bound steroid, and that the systemic component is of less importance.

Keywords: Allergy; *Ascaris suum*; bronchial circulation; budesonide; cysteinyl leukotrienes; histamine; late airways obstruction; sensitized minipigs

Introduction

It has long been known that glucocorticoids can inhibit late asthmatic reactions in human subjects (Booij-Noord *et al.*, 1971), and these substances are now frequently used in the treatment of asthma. To avoid systemic side-effects associated with glucocorticoid treatment, a glucocorticoid with a high ratio for topical over systemic activity would be preferable. Budesonide, with potent anti-inflammatory action (Brattsand *et al.*, 1982), shows high affinity for the lung and is not metabolized to any major extent within the lung (Ryrfeldt *et al.*, 1989). In contrast, budesonide is rapidly metabolized in the liver once absorbed to the systemic circulation (Ryrfeldt *et al.*, 1982). Pharmacokinetic properties like these would be expected to minimize systemic side-effects; however, it has been difficult to prove that the topical portion of inhaled glucocorticoids can be given full credit for the anti-inflammatory effect of glucocorticoids in asthma. One study (Toogood *et al.*, 1990) showed that inhaled budesonide, but not oral budesonide, had anti-asthmatic effects in human subjects, but plasma levels of budesonide were not measured in this study. Some studies performed in the dog have shown good anti-inflammatory effects of aerosolized budesonide (Stevens *et al.*, 1994; Woolley *et al.*, 1994a,b). However, in these studies rather high doses of inhaled budesonide were used and the effects were not compared with those of systemically administered steroid.

[1]Author for correspondence at: Division of Pharmacology, Department of Physiology and Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden.

A new large-animal model for studies of the late allergic reaction in the lower airways has recently been developed (Fornhem *et al.*, 1995a,b). *Ascaris suum* challenge in the lower airways of actively-sensitized, low cortisol (metyrapone-treated) pigs results in an acute increase in airways resistance, followed by a late increase starting at approximately 4 h after challenge. During the acute reaction, histamine and cysteinyl-leukotrienes (cys-LT) are released. In metyrapone-treated pigs, a continuous release of cys-LT is also seen during the late reaction. Blood flow in the bronchial artery, supplying the bronchial mucosa increases during the acute reaction and a late increase in blood flow is also seen, independent of the presence of late airways obstruction. The purpose of the present study was to determine the effects of exogenously administered steroid on allergen-induced late reactions in the pig. Minipigs were chosen to enable future chronic experiments, and the sensitization procedure was improved by using a highly allergenic protein fraction purified from an *A. suum* extract. In the sensitized minipig, we examined the effects of a single low dose of budesonide, administered as an aerosol or as an intravenous infusion, on changes in pulmonary mechanics and mediator release evoked by airway challenge with *A. suum*.

Methods

The experiments were approved by the local Ethical Committee for animal research.

Purification of A. suum *allergen*

Crude freeze-dried *A. suum* extract was dissolved in 0.075 M Tris, pH 7.4, ultracentrifuged 2 times for 1 h at 20,000 *g* and applied on a Sephacryl S-200 superfine column with a total volume of 500 ml (Pharmacia Biotech AB, Uppsala, Sweden) at a flow rate of 0.8 ml h^{-1}. The sample volume was 10 ml and 10 ml fractions were collected. Absorbance was measured at 280 nm and 260 nm on a Hitachi 150–20 spectrophotometer. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a PhastSystem (Pharmacia Biotech) under unreduced conditions using gels with 10–15% polyacrylamide and silver staining according to the manufacturer's instructions.

Sensitization

Twenty-three male specific pathogen-free Göttingen minipigs (Ellegaard, Dalmose, Denmark) weighing 11–23 kg were used in the study. All of them were sensitized with s.c. injections of 0.13 mg *A. suum* allergen purified from crude extract, in a suspension of $Al(OH)_3$ starting at the age of approximately 6 months. Booster doses were given after 3 and 6 weeks.

Surgical preparation

About one week after the last allergen booster, the pigs were fasted overnight and premedicated with ketamine hydrochloride (20 mg kg^{-1} i.m.) and anaesthesia was induced by sodium pentobarbitone (12 mg kg^{-1} i.v.) introduced into an ear vein (09 h 00 min). Blood samples for analysis of basal cortisol levels were taken from this vein. The adequacy of anaesthesia was tested by pinching the interdigital skin. Pancuronium bromide was given to achieve muscle relaxation (0.2 mg kg^{-1}, i.v.) and after tracheotomy, the pigs were intubated and ventilated with a mixture of air and oxygen using a Servo ventilator (900; Siemens-Elema, Sweden). No positive end-expiratory pressure was used. Anaesthesia was maintained by continuous i.v. infusion of pentobarbitone (9 mg kg^{-1} h^{-1}) and pancuronium bromide (0.6 mg kg^{-1} h^{-1}) through a catheter placed in one femoral vein. Ringer solution with 0.5% glucose (250 ml h^{-1}) was given through the same catheter. A femoral artery was cannulated for continuous recordings of mean arterial pressure (MAP) and heart rate, and the stability of these parameters was used as control of the adequacy of anaesthesia. Blood samples were drawn from a catheter in a femoral artery. Heparin was given in all catheters in a total of 4000 iu before the start of the experiment. Arterial blood gas partial pressures and pH were monitored at least hourly with an automatic blood gas analyser (IL 1302, Metric AB, Solna, Sweden). Arterial blood gases and pH were adjusted by changing the ventilator settings to a P_{O_2} of 12–15 kPa, a P_{CO_2} of 4.5–5.5 kPa and a pH of 7.4–7.5 before allergen challenge. Body temperature was maintained at 38–39°C with a heating pad connected to a thermostat. For urine collection, the ureter on the left side was dissected free and a catheter was inserted. After a right-side thoracotomy, the bronchial artery, which supplies the tracheobronchial tree from the lower trachea down to the peripheral bronchioles, was dissected free and a flow probe (Transonic probe 2SB) was placed around the vessel and connected to an ultrasonic blood flow meter (T202S; Transonic System Inc., Ithaca, NY, USA) for continuous recording of absolute blood flow. The resolution of the probe was 0.1 ml min^{-1} and the relative accuracy was ±2% according to the manufacturer. The respiratory pressure was measured by connecting an outlet of the extratracheal tube to a Stratham PM 131 TC pressure transducer and this value was used as a measure of transrespiratory pressure (P_{tr}), since intrapleural pressure was equal to atmospheric pressure because of the thoracotomy. Airflow ($\dot{V}$) was measured with a heated pneumotachygraph (Model 3500A, Hans Rudolph Inc., Kansas City, U.S.A.) connected to a pressure transducer (Kent Scientific, Litchfield, CT, U.S.A.).$\dot{V}$ and P_{tr} signals were sent to an AP 200 Pulmonary Computer (ConMeTech AB, Uppsala, Sweden) for on-line calculations of total lung resistance (R_L) and dynamic lung compliance (C_{dyn}). All cardiovascular and pulmonary parameters were continuously recorded on a Grass polygraph and simultaneously collected on an Apple Macintosh data acquisition system for analyses and graphical presentation.

Skin testing

A skin test using the crude extract of *A. suum* in a 10 fold dilution series was performed to classify the degree of sensitivity to the allergen. The allergen was given intradermally in a volume of 50 µl per site 1 h prior to the aerosol challenge. The end-point of titration was determined as the lowest concentration giving a dark reddening exceeding 2 mm, 10 min after the injection. Pigs responding only to undiluted extract were classified as class 1-responders, those responding to a 10 fold dilution were class 2, those responding to a 100 fold dilution class 3, etc. Pigs of classes 3 and higher were included in the study (one pig was excluded).

Metyrapone treatment

All pigs were given a bolus dose of metyrapone of 10 mg kg^{-1}, i.v., 2 h before the allergen challenge (about 11 h 00 min), followed by a continuous i.v. infusion (1.0 mg kg^{-1} h^{-1}).

Budesonide inhalation

Micronized dry powder of budesonide was compressed to a tablet in a brass cup supplied by Astra Draco AB (Lund, Sweden). The cups were connected to a modified Wright Dust Feeder (Adams, London, UK) driven by a speed-control motor (Motomatic II, Electro-Craft, South Eden Prairie, MN, U.S.A.). Powder was scraped off the tablet by a scraper blade and aerosolized in a current of air (airflow 10 l min^{-1}) measured by a rotameter (Fisher 1100). The aerosol was fed into the endotracheal tube. The particle concentration generated by the Wright Dust Feeder was monitored by a light-scattering instrument (model AMS 950, Casella, Bedford, U.K.) and the speed of the Wright Dust Feeder was adjusted if necessary to maintain a constant particle concentration predetermined to deliver the dose over 5 min inhalation. The particle size of

budesonide (1.86 μm; mass mean aerodynamic diameter, geometric s.d. 2.0) was measured with an aerodynamic particle sizer (model APS 33B, TSI, St. Paul, MN, U.S.A.) in a vertical wind tunnel.

Six pigs were given budesonide aerosol 1 h before allergen challenge in an amount of 10.2 ± 1.2 μg kg^{-1} (denoted budesonide aerosol group).

Budesonide infusion

Solubilized budesonide for the i.v. infusion was stored at 4°C at a concentration of 0.5 mg ml^{-1} in 10% ethanol and 40% polyethyleneglycol. The stock solution was diluted in saline immediately prior to the infusion. Five pigs (denoted budesonide infusion group) were given a budesonide infusion of 5 μg kg^{-1} during a 5 min period 1 h before allergen challenge in a femoral vein catheter with an outlet near the heart. Six pigs (denoted budesonide vehicle group) were instead given an infusion of diluted budesonide vehicle (0.4% ethanol and 1.5% polyethyleneglycol in saline) 1 h before allergen challenge.

Antigen challenge

Challenge with the nebulized allergen was performed 1.5 h after the end of the surgical preparation. The allergen consisted of an ultracentrifuged extract of *A. suum* delivered to an ultrasonic nebulizer (NB 108, Engström Medical, Stockholm, Sweden) connected to the inspiratory limb of the ventilator. The nebulizer generated particles with a diameter of 0.5–10 μm resulting in even distribution of the antigen in the airways. The aerosol was delivered via an endotracheal tube to the lower airways until an increase in tracheal pressure of 5 cmH_2O was achieved. The amount delivered to the aerosol chamber was 7–35 mg in 0.5–2.5 ml saline. The five control pigs were given 20 mg of ovalbumin in 2 ml saline as an aerosol during 5 min.

Budesonide tissue level measurement

The animals were killed with an overdose of pentobarbitone and central and peripheral lung tissue was immediately collected and frozen in dry-ice-chilled isopentane. The tissue was stored at −70°C until analysis of budesonide at Astra Draco AB. Frozen lung tissue was thawed and homogenized (Polytron PT3000, Kinematica AG, Switzerland) together with water. The homogenate was freeze-dried (Flexi-Dry MP, FTS Systems, Inc., U.S.A.). The freeze-dried samples were extracted in ethanol together with an internal standard in a microwave extracting system (MES 1000, CEM Corporation, NC, U.S.A.) at a temperature of 90°C for 30 min. The extracts were centrifuged at 1520 *g* for 15 min (Beckman TJ-6, Beckman Instruments Inc. Spinco Division, CA, U.S.A.). Budesonide levels in extracts were measured by liquid chromatography and mass spectrometry (Finnegan SSQ700 equipped with Thermospray interface, Finnegan Corporation, CA, U.S.A.).

Budesonide plasma level measurement

Heparinized venous blood (20 ml) was centrifuged at 750 *g* for 20 min at 4°C. The plasma was withdrawn and separated into aliquots of 3.5 ml and stored at −20°C. Budesonide levels were analysed at Astra Draco AB by mass spectrometry. Plasma cortisol did not interfere with the measurement of budesonide.

Arterial blood gases

Arterial blood for monitoring of blood gases and pH was drawn 45 and 5 min prior to allergen challenge and 15 min and once every hour after the allergen challenge. Supplemental oxygen was given to pigs that had severe acute reactions (i.e. if P_{O_2} was less than 7 kPa at 15 min). Pigs with severe acidosis (pH less than 7.25 at 15–30 min) were given a buffering solution (a combination of trometamol, bicarbonate and acetate; Tribonat) as an i.v. infusion until arterial pH was stabilized. No ventilatory corrections were made to compensate for acidosis. Between 2 and 8 h after *A. suum* challenge no more buffering solution was given, and supplementary oxygen was fixed at a certain level at 2 h.

Plasma levels of cortisol and catecholamines

Arterial blood mixed with EDTA to a final concentration of 10 mM was kept on ice for a maximum of 30 min before centrifugation at 4°C, 680 *g* for 10 min. Plasma was collected and stored at −70°C until analysis. Plasma concentrations of cortisol were determined by a radioimmunoassay (Orion Diagnostica AB, Trosa, Sweden), with a detection limit of 3–5 nM. In two blood samples, budesonide was added to a concentration of 23 μM to check for possible interference of budesonide in the cortisol radioimmunoassay. Catecholamines (adrenaline and noradrenaline) were determined after alumina extraction by cation-exchange high-performance liquid chromatography (h.p.l.c.) with electrochemical detection according to Hjemdahl (1987).

Detection of urinary mediators

Urine was collected on ice in 1 h fractions, starting 1 h prior to the allergen challenge. Samples were centrifuged for 10 min at 4°C and 680 *g* and stored at −70°C until analysed.

Methylhistamine was determined by radioimmunoassay (Pharmacia AB) after dilution of samples 300 times.

Immunoreactive LTE_4 equivalents were determined with radioimmunoassay using [^{3}H]-LTE_4 as a tracer and a monoclonal LTD_4-antibody with cross-reactivities for LTC_4 and LTE_4 of about 50% (Advanced Magnetics Inc., Cambridge, MA, U.S.A.). The standard curve was set up with synthetic LTE_4 and the detection limit was about 50 fmol. All reagents were incubated at 4°C overnight. The antigen-antibody complexes formed were precipitated with polyethyleneglycol, samples were centrifuged, and free radiolabelled ligand in the supernatant was counted with liquid scintillation.

Creatinine was determined by standard colorimetric assay by the alkaline picrate method (Sigma Diagnostics, St. Louis, MO, U.S.A.).

Calculations and statistics

Blood flow was recorded in ml min^{-1} and vascular resistance (VR) in the bronchial circulation was defined as MAP divided by bronchial blood flow.

R_L and C_{dyn} were calculated as described by Mead (1961). R_L was calculated using the formula

$$R_L = \frac{P_{tr1} - P_{tr2}}{\dot{V}_1 - \dot{V}_2}$$

at the 50% isovolumetric level. C_{dyn} was calculated as

$$\frac{\text{tidal volume}}{P_{tr\,max}}$$

The concentration of methylhistamine and LTE_4 in urine was divided by the concentration of creatinine in the same samples.

Data are presented as mean ± s.e. mean. Statistical evaluations were performed using Student's two-tailed *t* test (differences considered significant if $P < 0.05$) and one-way analysis of variance (ANOVA) (paired or unpaired) when appropriate. Plasma adrenaline and noradrenaline values were transformed to logarithmic values to obtain normal distribution. R_L and C_{dyn} were evaluated by the Mann-Whitney *U* test (non-parametric) due to differences in variation within groups.

Correlation statistics were performed using Pearson's cor-

relation test (parametric). Relative increase in R_L and decrease in C_{dyn} from baseline were used. All evaluations were performed using InStat (GraphPad Software) on an Apple Macintosh computer.

Drugs

A. suum extract (Pharmacia AB, Diagnostics, Uppsala, Sweden), ketamine hydrochloride (Parke-Davis, Barcelona, Spain), sodium pentobarbitone (Apoteksbolaget, Umeå, Sweden), pancuronium bromide (Organon, Oss, The Netherlands), metyrapone (Sigma, St. Louis, MO, U.S.A.), ovalbumin (Sigma) and Tribonat (Pharmacia AB) were obtained as indicated.

Results

Sensitization

Purification of the crude *A. suum* extract on the gel filtration column resulted in a chromatogram which, read at 280 nm, had a major peak at the void volume, containing large proteins and complexes, a peak containing a 15 kD protein and a peak eluted after one column volume and showing absorption at 260 nm. Proteins with a molecular weight ranging from 10 to 70 kD and eluted between 46–69% of the column volume were pooled and used for sensitization. The major protein in this pool was 15 kD, but bands at 25 and 50 kD were also noted. Fractions not included in the pool were those containing large complexes and proteins with molecular weight over 100 kD and fractions eluted after one column volume, mostly nucleic acids.

All pigs responded with an acute reddening and oedema to *A. suum* challenge in the skin. Pigs included in the study were of class 3 (1 pig), class 4 (8 pigs), class 5 (5 pigs), class 6 (6 pigs) and class 7 (2 pigs). The average skin sensitivity did not differ between the groups.

Budesonide plasma and tissue levels

Plasma budesonide concentration and the amount of budesonide found in lung tissue at 8 h are presented in Table 1.

Plasma budesonide concentration was significantly higher 1 h prior to *A. suum* challenge in pigs given budesonide as an infusion than in those given budesonide as an aerosol. The difference was smaller 30 min prior to *A. suum* challenge, and at 4 and 8 h after *A. suum* challenge, no differences could be detected between the two groups. The area under the plasma concentration curve for budesonide from budesonide administration 1 h before until 8 h after *A. suum* challenge was similar in the two groups (Table 1).

Lung mechanics

The basal levels of R_L and C_{dyn} at the time of *A. suum* challenge were similar in all 4 groups (Table 2). The mean amount of *A. suum* allergen delivered to the nebulizer was 22.1 ± 4.5 ($n = 17$) and did not differ between groups.

A. suum challenge caused an acute increase in R_L with a peak at 12–13 min which had partially resolved at about 1 h (Figure 1). The acute reaction was followed by a further late increase in R_L in the budesonide vehicle and budesonide infusion groups (starting at 3.8 ± 0.6 and 1.9 ± 0.7 h after allergen challenge, respectively). No late increase in R_L was seen in the budesonide aerosol group. R_L was significantly different in the budesonide aerosol and budesonide infusion groups between 2 and 8 h after allergen challenge ($P < 0.05$, Mann-Whitney U test, $n = 5-6$). In the ovalbumin-challenged group, no changes whatsoever in R_L were seen.

After *A. suum* challenge there was a fall in C_{dyn} that peaked at 8–16 min and returned partially to baseline within 1 h (Figure 2). Between 2 and 8 h, C_{dyn} progressively decreased in all groups, including the ovalbumin-challenged pigs, with no significant difference between the *A. suum*-challenged pigs. However, the decrease in C_{dyn} in the budesonide vehicle group was significantly larger than in the ovalbumin group, whereas the development of C_{dyn} in budesonide-treated pigs was closer to that seen in ovalbumin-challenged pigs.

Plasma cortisol and catecholamines

Budesonide did not interfere with the measurement of plasma cortisol. Plasma cortisol levels 15 min prior to allergen challenge and 8 h after challenge were not influenced by budesonide treatment (Table 3). Cortisol levels decreased during the experiment in all groups of pigs.

No differences in plasma levels of catecholamines were found between the different groups 15 min prior to allergen challenge (Table 3). Adrenaline levels were significantly elevated in the budesonide infusion group and noradrenaline levels were significantly elevated in the budesonide vehicle group 8 h after allergen challenge, whereas none of the catecholamines was increased in the budesonide aerosol or ovalbumin groups (Table 3).

Arterial blood gases and pH

Arterial blood gases and pH before the allergen challenge (−15 min), during the acute reaction (+15 min), after the acute reaction had resolved (+2 h) and at 8 h after challenge are presented in Table 4. No differences in basal levels (−15 min) between the 4 groups in arterial blood gases and

Table 1 Plasma concentration of budesonide (nM), area under the budesonide plasma concentration curve (AUC) after budesonide infusion or aerosol administration (1 h before allergen challenge) until the end of the experiment 8 h after challenge (nM h), and lung tissue content of budesonide at the end of the experiment (pmol g^{-1} dry tissue)

Time before or after A. suum *challenge*	*Budesonide infusion* (5 μg kg^{-1}) *plasma*	*lung*	*Budesonide aerosol* (10 μg kg^{-1}) *plasma*	*lung*
−1 h[$]	14.6 ± 1.2***		5.86 ± 0.82	
−30 min	1.95 ± 0.18*		3.03 ± 0.44	
+4 h	0.28 ± 0.04		0.29 ± 0.04	
+8 h	0.16 ± 0.03	18.4 ± 3.5**	0.16 ± 0.02	45.2 ± 4.9
AUC	10.3 ± 1.2		11.4 ± 1.2	

[$] Blood samples taken immediately after the end of budesonide administration. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to the aerosol group, Student's t test, $n = 5-6$.

Table 2 Basal levels of R_L ($cmH_2O l^{-1} s$), C_{dyn} (ml cmH_2O^{-1}), MAP (mmHg), heart rate (b.p.m.) and vascular resistance in the bronchial circulation (VR_{Br}) (mmHg ml^{-1} min) at the time of allergen challenge

	Budesonide vehicle	*Budesonide infusion*	*Budesonide aerosol*	*Ovalbumin*
R_L	4.1 ± 0.9	3.6 ± 0.2	4.4 ± 0.8	4.9 ± 0.7
C_{dyn}	15.5 ± 2.2	17.4 ± 0.6	19.6 ± 1.8	17.1 ± 2.6
MAP	123 ± 7	128 ± 5	135 ± 6	138 ± 7
Heart rate	113 ± 10	113 ± 6	130 ± 9	125 ± 17
VR_{Br}	27.1 ± 4.5	59.4 ± 34.6	12.4 ± 4.9	23.1 ± 5.1
Methylhistamine	0.62 ± 0.12	0.57 ± 0.07	0.35 ± 0.02	0.45 ± 0.16
LTE_4	0.81 ± 0.38	0.89 ± 0.16	1.39 ± 0.26	0.55 ± 0.19

The basal levels of the mediators methylhistamine (mmol mol^{-1} creatinine) and LTE_4 (μmol mol^{-1} creatinine) are from urine collected during the hour prior to allergen challenge. No significant difference between the groups was noted for any parameter (ANOVA, $n = 5-6$).

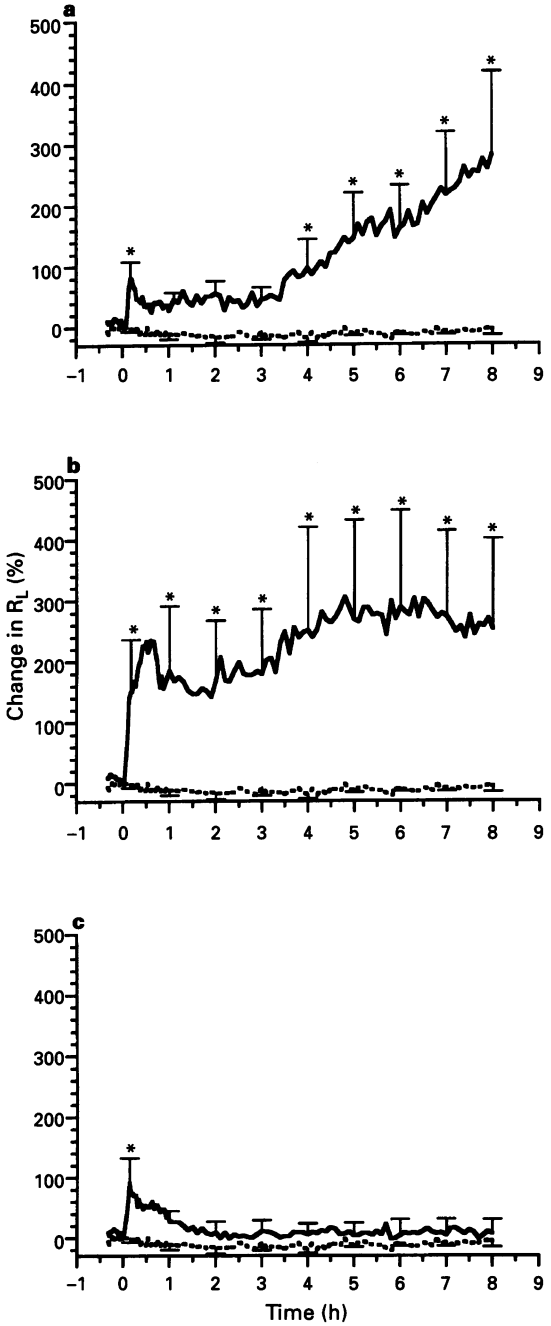

Figure 1 Changes in R_L in (a) budesonide vehicle ($n=6$), (b) budesonide infusion ($n=5$) and (c) budesonide aerosol ($n=6$) pigs. The *A. suum*-challenged pigs (continuous lines) are compared to ovalbumin-challenged pigs (broken lines, $n=5$), $^*P<0.05$, Mann-Whitney *U* test.

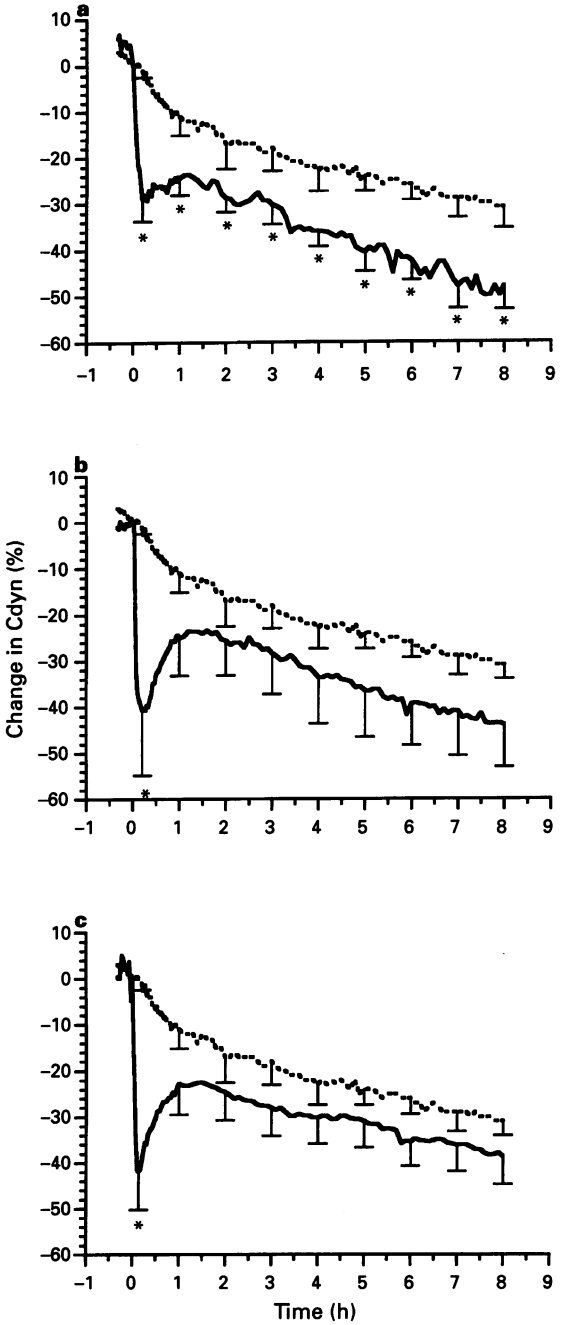

Figure 2 Changes in C_{dyn} in (a) budesonide vehicle ($n=6$), (b) budesonide infusion ($n=5$) and (c) budesonide aerosol ($n=6$) pigs. The *A. suum*-challenged pigs (continuous lines) are compared to ovalbumin-challenged pigs (broken lines, $n=5$), $^*P<0.05$, Mann-Whitney *U* test.

pH were found (ANOVA). During the acute reaction, there was a fall in P_{O_2} and pH and a rise in P_{CO_2} in the *A. suum*-challenged groups. During the late reaction, P_{O_2} was still markedly decreased in the budesonide vehicle group, but not in the budesonide aerosol and budesonide infusion groups. The late decrease in P_{O_2} was seen even though $28\pm2\%$ oxygen was given from 2 to 8 h after *A. suum*-challenge (no differences between groups), whereas pigs challenged with ovalbumin did not receive supplementary oxygen.

Arterial blood P_{CO_2} in *A. suum*-challenged pigs progressively increased in the budesonide vehicle and budesonide infusion groups, whereas in the budesonide aerosol group P_{CO_2} started to return to baseline within the observation period. A similar pattern was seen for arterial pH, and at 8 h, the pH in the budesonide infusion group was lower than the pH in the budesonide aerosol group ($P<0.05$, unpaired *t* test, $n=5-6$). Buffer solution was given intravenously during the acute reaction to none of the budesonide vehicle, 3 of the budesonide infusion and 4 of the budesonide aerosol pigs to prevent extreme acidosis. Blood gas values and pH were stable within the observation period in the ovalbumin group, except for a slight decrease in P_{O_2} at 8 h.

Mean arterial pressure and heart rate

MAP and heart rate at 15 min before allergen challenge were not statistically different in the four groups of pigs (see Table 2) with overall MAP and heart rate values of 130 ± 4 mmHg and 119 ± 4 beats per min ($n=22$), respectively. An acute increase in both MAP ($25.9\pm4.9\%$) and heart rate

(49.2±10.7%) was noted in all *A. suum*-challenged pigs ($n=17$) with a peak after approximately 10 min. No acute changes in MAP or heart rate were seen in ovalbumin-challenged pigs. The increase in MAP had resolved within 1 h, and from this time and to the end of the experiment, MAP progressively decreased. This decrease was more pronounced in budesonide vehicle pigs and budesonide infusion pigs than in budesonide aerosol pigs, reaching levels of 78±5 ($n=6$), 75±5 ($n=5$) and 95±4 ($n=6$) mmHg at 8 h, respectively. The decrease in MAP in the ovalbumin-challenged pigs was small (reaching 112±4 mmHg at 8 h, $n=5$). The increase in heart rate had not resolved completely at 1 h after *A. suum* challenge and was still slightly elevated in budesonide vehicle pigs and budesonide infusion pigs 8 h after *A. suum* challenge (156±19 and 148±21 mmHg, respectively, $n=5-6$), but not in budesonide aerosol pigs and ovalbumin-challenged animals (121±10 and 116±9 mmHg, respectively, $n=5-6$).

Vascular resistance in the bronchial circulation

Vascular resistance in the bronchial circulation was successfully measured in 15 of the pigs used in the study. Basal values are shown in Table 4. An acute decrease in vascular resistance was noted in the *A. suum*-challenged pigs. After the first decrease, vascular resistance first returned towards basal values and then decreased again with different time course in individual pigs. This second decrease was observed irrespective of whether or not late bronchial obstruction was present and budesonide treatment did not influence these changes in bronchial vascular resistance (Figure 3).

Mediators in urine

Basal levels of the urinary metabolites methylhistamine and leukotriene E_4 (LTE_4) are listed in Table 2. A significant increase in urinary methylhistamine was noted in the budesonide infusion and budesonide aerosol groups during the acute reaction (Figure 4). The increase in the budesonide vehicle group was moderate and was significant only in urine collected during the second hour after allergen challenge. No increase in methylhistamine was seen during the late reaction in any group. LTE_4 was increased in some of the pigs during the acute reaction, resulting in significant increases of LTE_4 in the budesonide-treated pigs in urine collected during the second hour after allergen challenge (Figure 5). No significant increase in LTE_4 levels could be detected during the late reactions in the *A. suum*-challenged groups, although 2 and 3 animals showed increased levels between 4 and 8 h in the budesonide vehicle and budesonide infusion groups, respectively. In the budesonide aerosol group none of the pigs showed this increase.

Table 3 Plasma concentration of cortisol, adrenaline and noradrenaline at 15 min prior to allergen challenge and at the end of the experiment (8 h after challenge)

	Budesonide vehicle	*Budesonide infusion*	*Budesonide aerosol*	*Ovalbumin*
Cortisol				
−15 min	50.4±9.8	61.2±7.1	75.5±4.8	91.9±19.5
8 h	23.2±4.4†	23.4±1.2†	22.8±0.9†	25.8±1.7†
Adrenaline				
−15 min	2.1±1.1	1.0±0.2	1.2±0.4	1.6±0.4
8 h	2.8±0.7	4.5±1.7†	1.4±0.5	1.5±0.7
Noradrenaline				
−15 min	1.9±0.8	3.1±1.4	1.9±0.6	1.7±0.2
8 h	20.3±11.5†	12.2±5.3	2.8±0.8	1.3±0.2

All concentrations are given in nM. †$P<0.05$ compared to 15 min before challenge (paired t test). The basal levels (−15 min) or the levels at 8 h were not different in the 4 groups (ANOVA, $n=5-6$).

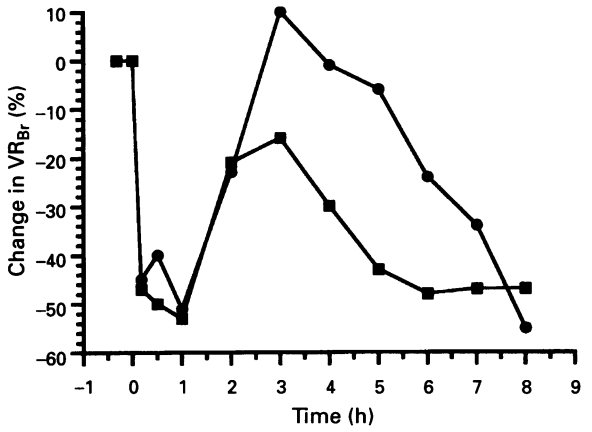

Figure 3 Changes from basal levels in vascular resistance in the bronchial circulation (VR_{Br}) in a budesonide infusion pig (●) receiving late bronchial obstruction and a budesonide aerosol pig (■) without late bronchial obstruction. Similar vascular responses were also seen in budesonide vehicle pigs.

Table 4 Arterial blood oxygen tension (P_{O_2}, kPa), carbon dioxide tension (P_{CO_2}, kPa) and pH at different time points from allergen challenge

	Budesonide vehicle	*Budesonide infusion*	*Budesonide aerosol*	*Ovalbumin*
P_{O_2}				
−15 min	14.6±1.1	13.3±0.5	13.7±0.5	13.2±0.4
15 min	7.2±0.8††	7.1±1.3††	7.3±1.0††	13.3±0.2
2 h	9.2±0.5††	10.5±0.7††	10.2±0.5†	12.4±0.5
8 h	8.1±1.0††	10.5±1.3	11.8±0.9	11.2±0.7
P_{CO_2}				
−15 min	5.0±0.3	5.2±0.3	5.3±0.2	5.1±0.2
15 min	6.0±0.2††	6.7±0.6†	6.7±0.4†	5.2±0.1
2 h	6.1±0.2††	6.9±0.8†	6.9±0.5†	5.1±0.3
8 h	7.6±1.0†	8.4±1.9†	6.4±0.4†	5.4±0.2
pH				
−15 min	7.44±0.03	7.45±0.02	7.43±0.01	7.45±0.02
15 min	7.38±0.02†	7.33±0.06†	7.36±0.02†	7.45±0.02
2 h	7.36±0.01	7.34±0.03†	7.34±0.03†	7.44±0.02
8 h	7.25±0.07†	7.27±0.05††	7.37±0.01†	7.44±0.01

†$P<0.05$ and ††$P<0.01$ compared to 15 min before allergen challenge (paired t test, $n=5-6$).

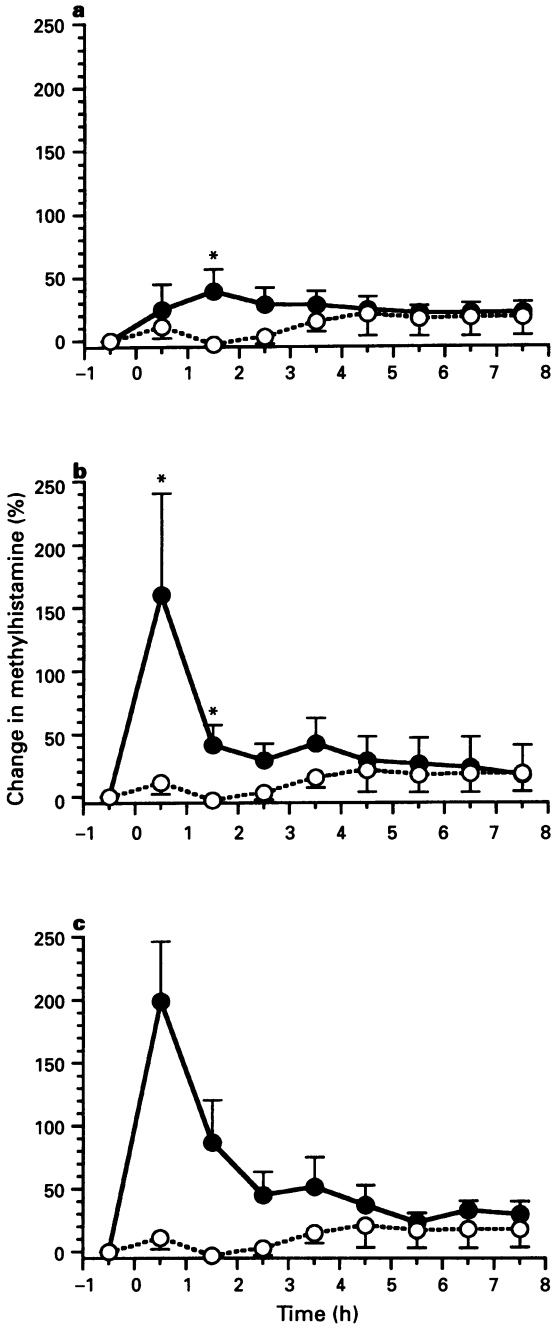

Figure 4 Changes in urinary methylhistamine in relation to creatinine excretion in (a) budesonide vehicle ($n=6$), (b) budesonide infusion ($n=5$) and (c) budesonide aerosol ($n=6$) pigs. The *A. suum*-challenged pigs (continuous lines) are compared to ovalbumin-challenged pigs (broken lines, $n=5$), $^*P<0.05$, Mann-Whitney *U* test. Symbols are placed in the middle of collecting periods (1 h).

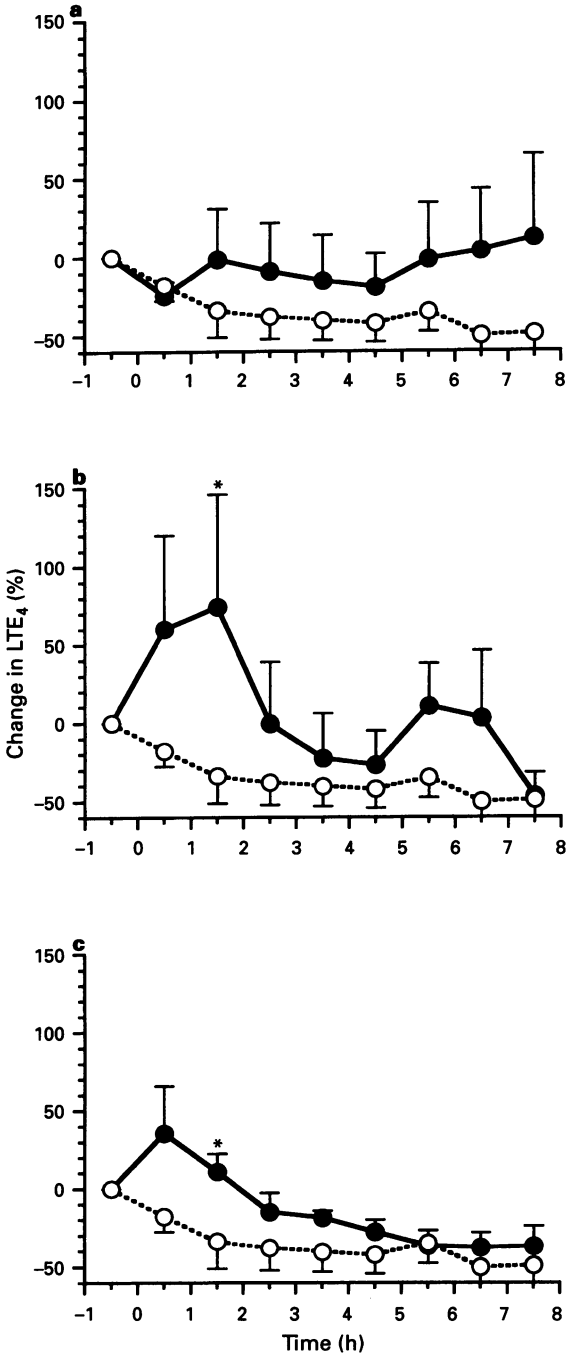

Figure 5 Changes in urinary leukotriene E_4 (LTE_4) in relation to creatinine excretion in (a) budesonide vehicle ($n=6$), (b) budesonide infusion ($n=5$) and (c) budesonide aerosol ($n=6$) pigs. The *A. suum*-challenged pigs (continuous lines) are compared to ovalbumin-challenged pigs (broken lines, $n=5$), $^*P<0.05$, Mann-Whitney *U* test. Symbols are placed in the middle of the collecting periods (1 h).

Correlations

The correlations (Pearson's correlation test) were made using all *A. suum*-challenged pigs. Budesonide in plasma correlated negatively with the late decrease in C_{dyn} ($P<0.05$, $r=-0.67$, $n=12$) whereas budesonide in lung tissue correlated negatively with the late increase in R_L ($P<0.05$, $r=-0.53$, $n=10$), but not *vice versa*. Acutely released methylhistamine correlated with acute changes in both R_L and C_{dyn} ($P<0.05$, $r=0.57$, $n=17$ and $P<0.01$, $r=0.66$, $n=17$, respectively). Acutely released LTE_4 correlated with the acute R_L response ($P<0.001$, $r=0.75$, $n=17$), but not with late responses in R_L or C_{dyn} or acute response in C_{dyn}.

Discussion

In this study the effects of locally and systemically administered budesonide on allergen-induced late reactions in the pig lower airways were studied. Budesonide was given in a clinically relevant dose delivered in two different ways 1 h before allergen challenge. The amount of budesonide administered was adjusted to give the same area under the curve for plasma concentrations of budesonide after local and systemic administration.

To improve the sensitization procedure, which entails subcutaneous injections of *A. suum* extract in aluminium hydroxide, allergenic proteins were purified from the crude

extract. Some components from the *A. suum* extract have been shown to suppress the immunoglobulin E (IgE) response to other allergens (Soares *et al.*, 1992). The immunosuppressive components seem to be complexes with high molecular weight (approximately 500 kD), while the highest IgE titres against *A. suum* were found after immunization with proteins of approximately 29 kD and in the present study proteins with molecular weight of 10–70 kD were used for the sensitization procedure. When the purified allergenic fraction was used for sensitization, skin testing revealed that both minipigs (present study) and domestic pigs (unpublished data) were markedly more sensitive in the skin than pigs sensitized with the crude extract. It has been suggested that the anti-inflammatory effect of budesonide is locally mediated (Brogden & McTavish, 1992). However, few studies have been performed to confirm this (Toogood *et al.*, 1990). Budesonide is a glucocorticoid with high affinity to lung tissue (Brattsand *et al.*, 1982); it has high intrinsic activity and is metabolized rapidly in the liver predominantly by oxidative metabolism (Brattsand *et al.*, 1982). Budesonide is not metabolized in the lung to any major extent (Brattsand *et al.*, 1982; Ryrfeldt *et al.*, 1989). The absorption to the circulation of aerosolized budesonide powder administered to the lung was very rapid in the pig, with maximal plasma levels 5 min after the end of the aerosol, as seen also in man (Ryrfeldt *et al.*, 1982). In the present study, the doses of budesonide were chosen to represent approximately the same amount of budesonide whether given as an aerosol or by the intravenous route. Studies performed in dogs in an experimental system similar to ours showed that approximately 46% of the nebulized budesonide was deposited in the lung (Woolley *et al.*, 1994b). We, thus, chose to give aerosolized budesonide at 10 $\mu g\ kg^{-1}$ and intravenous infusion at 5 $\mu g\ kg^{-1}$ to achieve similar plasma concentrations. The calculated area under the budesonide plasma concentration curve during the experiment (−1 to 8 h) showed that the same amount of budesonide reached the circulation when given by aerosol and by infusion, whereas the amount of tissue-bound budesonide was higher in the aerosol group.

The use of glucocorticoids may inhibit the release of adrenocorticotropic hormone resulting in lower levels of plasma cortisol (Johansson *et al.*, 1982). However, in our study, when both control animals and experimental subjects were treated with the cortisol-synthesis inhibitor metyrapone, no effects of the exogenous glucocorticoid administration on plasma cortisol levels could be seen.

Judging from the changes in lung mechanics, and the release of histamine and cys-LTs, the acute reaction after *A. suum* challenge was more pronounced in the pigs treated with budesonide. This indicates that glucocorticoids may affect IgE-mediated mast cell activation. One possible mechanism could be that steroids decrease the production of the proposed mast cell inhibitor, prostaglandin E_2 (Raud *et al.*, 1988), resulting in more powerful release of mediators in pigs with higher levels of steroids. This is also supported by other studies that showed trends towards e.g. higher release of mast cell mediators from dexamethasone-treated human lung tissue (Schleimer *et al.*, 1986), and weaker antigen-induced acute bronchoconstriction in metyrapone-treated rats (Turner *et al.*, 1994), dogs (Sasaki *et al.*, 1987) and pigs (Fornhem *et al.*, 1995b). Also, pretreatment of sensitized pigs with the cyclo-oxygenase blocker, diclofenac, markedly enhances the acute bronchoconstriction seen after *A. suum* challenge (Alving *et al.*, 1991b), further indicating the inhibitory role of prostaglandins in airway allergic reactions. However, in spite of relatively moderate acute reactions in the budesonide vehicle group, a severe late reaction was seen, starting at 3–4 h after allergen challenge. This late reaction consisted of a bronchial obstruction, and impairment of arterial blood gases and pH, similar to the reaction described earlier in the pig (Fornhem *et al.*, 1995b). No sign of stress as measured by catecholamine levels at 8 h was seen in ovalbumin or budesonide aerosol pigs, whereas late bronchial obstruction in budesonide vehicle and budesonide infusion groups was associated with a stress reaction at this time point.

Aerosolized budesonide given in a clinically relevant dose 1 h prior to allergen challenge inhibited the allergic late reaction in the pig as judged by the attenuation of changes in R_L and C_{dyn} blood gases, pH and plasma catecholamine levels seen within 8 h in this model. The rapid onset of the protective effect of budesonide in the airways has also been shown in human subjects, where budesonide inhaled at the start of late asthmatic responses is able to reduce the magnitude of this response (Paggiaro *et al.*, 1994). However, it seems important to reach a threshold steroid concentration, since halving of the budesonide aerosol dose in the pig resulted in almost no protection against late changes in R_L (unpublished data). LTE_4, the end metabolite for cys-LTs in the pig (Fornhem *et al.*, 1995a), which was detected in urine during the late reaction in some of the pigs in the budesonide vehicle and budesonide infusion groups, could not be detected in any animal in the budesonide aerosol group, suggesting a suppression of LTE_4 excretion when budesonide is given locally. Late release of cys-LTs after allergen challenge in the pig has been shown earlier and this release was shown to be sensitive to high plasma cortisol levels (Fornhem *et al.*, 1995a). However, due to large variations, no statistically significant difference was achieved in this study.

The progressive decrease in C_{dyn} was inhibited to a small extent by both aerosolized and infused budesonide. The plasma levels of budesonide correlated negatively with the late decrease in C_{dyn}. This was not seen for the concentration of budesonide in lung tissue, which correlated negatively with the late increase in R_L, but not with the late decrease in C_{dyn}. Infused budesonide shows much larger deposition in lung parenchyma compared to airways, whereas aerosolized budesonide is equally distributed in the two compartments (Dahlbäck *et al.*, 1994). Thus, circulating budesonide will relate more closely to effects in the pulmonary circulation (as reflected by C_{dyn}), whereas tissue-bound budesonide, as measured in both compartments together, will relate relatively more to effects in the airways (as reflected by R_L). The possible effect of budesonide on the pulmonary circulation could be due to the ability of budesonide to reduce leakage of plasma across both endothelial and epithelial barriers (Erjefält & Persson, 1986), which will lead to a reduction of the fall in C_{dyn}. Most of the decrease in C_{dyn} is probably caused by unspecific pulmonary leakage in this model using thoracotomized, metyrapone-treated pigs (Fornhem *et al.*, 1995b).

Interestingly, the biphasic course of the changes in vascular resistance in the bronchial circulation (Alving *et al.*, 1991a) was not altered by budesonide aerosol, whereas the decrease in MAP and the increase in heart rate during the late reaction was reduced in the budesonide aerosol pigs, which also lacked late bronchial obstruction. This suggests that the late bronchial vasodilatation is independent of late airways obstruction and systemic cardiovascular changes. Furthermore, the late vasodilatation seems to be highly resistant to glucocorticoid activity, even at high levels of endogenous cortisol (Fornhem *et al.*, 1995b). The mechanism for the late changes in bronchial blood flow remains to be determined.

The present data indicate that the late allergic reaction in the airways is a local phenomenon, as recently proposed by Dandurand *et al.* (1994), who saw an allergen-induced late bronchoconstrictor response in rat isolated lung explants *in vitro*. Despite the total inhibition of the late airways obstruction in the budesonide aerosol group, clear-cut eosinophil infiltration and activation were still seen in this group although slightly reduced compared to the budesonide vehicle group (Fornhem *et al.*, 1996). This suggests that the eosinophil is not the primary cause of the late airways obstruction. Instead, an important effect of budesonide in this model could be down-regulation of a cell already present in the lung, e.g. the macrophage, epithelial cell or T-lymphocyte.

In conclusion, our findings demonstrate that low doses of locally administered budesonide effectively block the late bronchial obstruction after allergen challenge in the pig, while budesonide infused in a dose resulting in the same plasma

concentration as after aerosol administration was ineffective on this reaction. This indicates that the late asthmatic reaction is to a major extent mediated by steroid-sensitive locally produced factors.

We thank M. Stensdotter and C. Nihlén at the Department of Physiology and Pharmacology, Karolinska Institute for expert technical and laboratory assistance. The authors are also indebted to Ola Nerbrink (aerosol expert), Alf Carlshaf (tissue extraction) and Mona-Lisa Kristensson (budesonide analysis) Astra Draco AB Lund.

This study was supported by grant from Astra Draco AB, the Swedish Association against Asthma and Allergy, the Swedish Medical Research Council (project Nos 10162, 10354 and 9071), the AGA AB Medical Research Fund, the Åke Wiberg Foundation, the Swedish Enviromental Protection Board, the Swedish Society of Medical Research, Pharmacia AB, the Swedish Heart-Lung Foundation, and the Karolinska Institute.

References

ALVING, K., MATRAN, R., FORNHEM, C. & LUNDBERG, J.M. (1991a). Late phase bronchial and vascular responses to allergen in actively-sensitized pigs. *Acta Physiol. Scand.*, **143,** 137–138.

ALVING, K., MATRAN, R. & LUNDBERG, J.M. (1991b). The possible role of prostaglandin D_2 in the long-lasting airways vasodilatation induced by allergen in the sensitized pig. *Acta Physiol. Scand.*, **143,** 93–103.

BOOIJ-NOORD, H., ORIE, N.G.M. & DE VRIES, K. (1971). Immediate and late obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J. Allergy Clin. Immunol.*, **48,** 344–354.

BRATTSAND, R., THALÉN, A., ROEMPKE, K., KÄLLSTRÖM, L. & GRUVSTAD, E. (1982). Development of new glucocorticosteroids with a very high ratio between topical and systemic activities. *Eur. J. Respir. Dis. Suppl.*, **122,** 62–73.

BROGDEN, R.N. & MCTAVISH, D. (1992). Budesonide. An updated review of its pharmacological properties, and therapeutic efficacy in asthma and rhinitis. *Drugs*, **44,** 375–407.

DAHLBÄCK, M., MATTSSON, H., PRYTZ, M., HJERTBERG, E., TUNEK, A., MILLER-LARSON, A. & BRATTSAND, R. (1994). Uptake of a glucocorticoid, budesonide, in rat airways and lung after inhalation and intravenous infusion. *Eur. Respir. J.*, **7,** 381s.

DANDURAND, R.J., WANG, C.G., LABERGE, S., MARTIN, J.G. & EIDELMAN, D.H. (1994). *In vitro* allergic bronchoconstriction in the Brown Norway rat. *Am. J. Respir. Crit. Care Med.*, **149,** 1499–1505.

ERJEFÄLT, I. & PERSSON, C.G.A. (1986). Anti-asthma drugs attenuate inflammatory leakage of plasma into airway lumen. *Acta Physiol. Scand.*, **128,** 653–654.

FORNHEM, C., KUMLIN, M., LUNDBERG, J.M. & ALVING, K. (1995a). Allergen-induced late-phase airways obstruction in the pig: mediator release and eosinophil recruitment. *Eur. Respir. J.*, **8,** 1100–1109.

FORNHEM, C., LUNDBERG, J.M. & ALVING, K. (1995b). Allergen-induced late-phase airways obstruction in the pig: the role of endogenous cortisol. *Eur. Respir. J.*, **8,** 928–937.

FORNHEM, C., PETERSON, C.G.B., DAHLBÄCK, M., SCHEYNIUS, A. & ALVING, K. (1996). Granulocyte function in the airways of allergen-challenged pigs: Effects of inhaled and systemic budesonide. *Clin. Exp. Allergy* (in press).

HJEMDAHL, P. (1987). Catecholamine measurements in plasma by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol.*, **142,** 521–534.

JOHANSSON, S.-A., ANDERSSON, K.-E., BRATTSAND, R., GRUVSTAD, E. & HEDNER, P. (1982). Topical and system glucocorticoid potencies of budesonide, beclomethasone dipropionate and prednisolone in man. *Eur. J. Respir. Dis. Suppl.*, **122,** 74–82.

MEAD, J. (1961). Mechanical properties of lungs. *Physiol. Rev.*, **41,** 281–330.

PAGGIARO, P.L., DENTE, F.L., MORELLI, M.C., BANCALARI, L., FRANCO, A.D., GIANNINI, D., VAGAGGINI, B., BACCI, E., FABBRI, L.M. & GIUNTINI, C. (1994). Postallergen inhaled budesonide reduces late asthmatic response and inhibits the associated increase of airway responsiveness to methacholine in asthmatics. *Am. J. Respir. Crit. Care. Med.*, **149,** 1447–1451.

RAUD, J., DAHLÉN, S.-E., SYDBOM, A., LINDBOM, L. & HEDQVIST, P. (1988). Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E_2: Apparent correlation with *in vivo* modulation of mediator release. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 2315–2319.

RYRFELDT, Å., ANDERSSON, P., EDSBÄCKER, S., TÖNNESSON, M., DAVIES, D. & PAUWELS, R. (1982). Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur. J. Respir. Dis. Suppl.*, **122,** 86–95.

RYRFELDT, Å, PERSSON, G. & NILSSON, E. (1989). Pulmonary deposition of the potent glucocorticoid budesonide, evaluated in an isolated perfused rat lung model. *Biochem. Pharmacol.*, **38,** 17–22.

SASAKI, H., YANAI, M., SHIMURA, S., OKAYAMA, H., AIKAWA, T., SASAKI, T. & TAKISHIMA, T. (1987). Late asthmatic response to *Ascaris* antigen challenge in dogs treated with metyrapone. *Am. Rev. Respir. Dis.*, **136,** 1459–1465.

SCHLEIMER, R.P., DAVIDSON, D.A., LICHTENSTEIN, L.M. & ADKINSON, JR, N.F. (1986). Selective inhibition of arachidonic acid metabolite release from human lung tissue by antiinflammatory steroids. *J. Immunol.*, **136,** 3006–3011.

SOARES, M.F.M., MOTA, I. & MACEDO, M.S. (1992). Isolation of *Ascaris suum* components which suppress IgE antibody responses. *Int. Arch. Allergy Immunol.*, **97,** 37–43.

STEVENS, W.H.M., ÄDELROTH, E., WATTIE, J., WOOLLEY, M.J., ELLIS, R., DAHLBÄCK, M. & O'BYRNE, P. (1994). Effect of inhaled budesonide on ozone-induced airway hyperresponsiveness and bronchoalveolar lavage cells in dogs. *J. Appl. Physiol.*, **77,** 2578–2583.

TOOGOOD, J.H., FRANKISH, C.W., JENNINGS, B.H., BASKERVILLE, J.C., BORGA, O., LEFCOE, N.M. & JOHANSSON, S.-A. (1990). A study of the mechanism of the antiasthmatic action of inhaled budesonide. *J. Allergy Clin. Immunol.*, **85,** 872–880.

TURNER, D.J., MYRON, P. & MARTIN, J.G. (1994). The role of endogenous corticosterone in the late response to allergen challenge in the Brown Norway (BN) rat. *Am. J. Respir. Crit. Care Med.*, **149,** A531.

WOOLLEY, M.J., DENBURG, J.A., ELLIS, R., DAHLBÄCK, M. & O'BYRNE, P.M. (1994a). Allergen-induced changes in bone marrow progenitors and airway responsiveness in dogs and the effect of inhaled budesonide on these parameters. *Am. J. Respir. Cell Mol. Biol.*, **11,** 600–606.

WOOLLEY, M.J., WATTIE, J., ELLIS, R., LANE, C.G., STEVENS, W.H.M., WOOLLEY, K.L., DAHLBÄCK, M. & O'BYRNE, P.M. (1994b). Effect of an inhaled corticosteroid on airway eosinophils and allergen-induced airway hyperresponsiveness in dogs. *J. Appl. Physiol.*, **77,** 1303–1308.

(Received August 30, 1995
Revised January 18, 1996
Accepted February 23, 1996)