Eur Respir J, 1995, 8, 928–937
DOI: 10.1183/09031936.95.08060928
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Allergen-induced late-phase airways obstruction in the pig: the role of endogenous cortisol

C. Fornhem, J.M. Lundberg, K. Alving

Allergen-induced late-phase airways obstruction in the pig: the role of endogenous cortisol. C. Fornhem, J.M. Lundberg, K. Alving. ©ERS Journals Ltd 1995.

ABSTRACT: In developing a novel model for studies of the allergen-induced late-phase airways obstruction, by using the pig, the importance of endogenous cortisol levels was examined by the use of metyrapone, a cortisol-synthesis inhibitor.

Specific-pathogen free pigs were actively sensitized with *Ascaris suum* allergen. One group of pigs was treated with a constant infusion of metyrapone in order to maintain low levels of plasma cortisol. *Ascaris suum* allergen was nebulized into the lower airways and plasma cortisol and catecholamine levels, total lung resistance and dynamic compliance, blood gases and pH, and blood flow in the bronchial circulation were continuously recorded for 8 h.

At the time of allergen challenge, the plasma cortisol levels in sensitized pigs were 455±37 nM and 40.1±3.8 nM in non-metyrapone-treated and metyrapone-treated pigs, respectively. No difference was seen between the magnitude of the acute bronchoconstrictor response in the two groups. A late airways obstruction, starting at about 4 h, developed only in pigs with low cortisol levels, whereas a late increase in blood flow in the bronchial circulation was seen in both groups, even if a late airways obstruction was absent. Plasma adrenaline did not seem to influence the late-phase reaction.

These results suggest that endogenous cortisol levels, but not adrenaline, modify the late response to allergen in the pig. Furthermore, it is suggested that the pig is a suitable model for studies of allergic reactions in the airways, if metyrapone is used to keep plasma cortisol levels within a normal range.

Eur Respir J., 1995, 8, 928–937.

Division of Pharmacology, Dept of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden.

Correspondence: C. Fornhem
Division of Pharmacology
Dept of Physiology and Pharmacology
Karolinska Institute
S-171 77 Stockholm
Sweden

Keywords: Airways obstruction, allergen, bronchial challenge, bronchial circulation, catecholamines, cortisol

Received: July 12 1994
Accepted after revision January 7 1995

This study was supported by grants from the Swedish Medical Research Council (project No. 10162 and 10354), the AGA AB Medical Research Fund, the Åke Wiberg Foundation, the Swedish Environmental Protection Board, the Swedish Society of Medical Research, Pharmacia Diagnostics and the Karolinska Institute.

The late-phase reaction after allergen challenge in the lower airways has been intensively studied since BOOIJ-NOORD *et al.* [1] demonstrated a glucocorticoid-sensitive late-phase reaction in the lung. In parallel, it has been reported that nocturnal asthma, which may be a kind of late-phase reaction [2], can be prevented by intravenous infusion of cortisol [3], thus preventing the circadian decrease in cortisol levels during the night. The endogenous cortisol level could, thus, play a critical role in development of the late-phase asthmatic response. This could be studied by using a cortisol-synthesis inhibitor, such as metyrapone, which will decrease plasma cortisol levels [4, 5]. However, it has also been suggested that circadian changes in adrenaline levels could be responsible for the development of nocturnal exacerbations of asthma, since a decrease of this mediator with bronchodilator properties is seen at the same time as airways obstruction occurs [6, 7].

Several animal models that represent different features of asthma have been developed, but none of them shows all the characteristics of human asthma [8]. We have found that the pig may be a new alternative for studies of allergic reactions in the airways [9, 10]. The purpose of this study was to characterize the late-phase reaction in the pig, in terms of pulmonary airways obstruction and changes in bronchial blood flow during and after the acute reaction to allergen, and their relation to endogenous cortisol and catecholamine levels. Allergen challenge in the lower airways was performed in anaesthetized and mechanically-ventilated pigs that had been actively sensitized with *Ascaris suum* (*A. suum*) allergen. Total lung resistance (R_L) and dynamic compliance (C_{dyn}), mean arterial pressure (MAP), heart rate, bronchial blood flow, arterial pH and gas partial pressures, and plasma cortisol, adrenaline and noradrenaline were followed for 8 h after challenge.

Methods

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

Surgical preparation

Twenty five specific pathogen-free pigs (Seropig; Vallrums Lantbruks AB, Ransta, Sweden) of either sex

were used in the study. At the age of 3, 6 and 9 weeks, 17 of the pigs were sensitized with subcutaneous injections in increasing doses of 0.6–1.0 mg *A. suum* allergen (Pharmacia Diagnostics, Uppsala, Sweden) in a suspension of $Al(OH)_3$. Eight pigs served as control group and were not sensitized. About one week after the third injection (at a body weight of 25–30 kg), the pigs were fasted overnight and premedicated with ketamine hydrochloride (Parke-Davis, Barcelona, Spain; 20 mg·kg^{-1} *i.m.*), and anaesthesia was induced by sodium pentobarbitone (Apoteksbolaget, Umeå, Sweden; 12 mg·kg^{-1} *i.v.*) introduced into an ear vein (9 a.m.). Blood samples for analysis of basal cortisol levels were taken in this vein. The adequacy of anaesthesia was tested by pinching the interdigital skin. Pancuronium bromide was given to achieve muscle relaxation (Organon, Oss, The Netherlands; 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. Anaesthsia was maintained by continuous *i.v.* infusion of pentobarbitone (7–9 mg·kg^{-1}·h^{-1}) and pancuronium (0.6 mg·kg^{-1}·h^{-1}) through a catheter placed into 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 MAP and heart rate. All blood samples were drawn from a catheter placed in a brachial artery. Heparin was given in all catheters to a total of 4,000 IU before the start of the experiment. Arterial blood gas partial pressures and pH were regularly monitored and base excess calculated using an automatic blood gas analyser (IL 1302, Metric AB, Solna, Sweden). Blood gases and pH were adjusted by changing of ventilator settings to an arterial oxygen tension (P_{a,O_2}) of 12–15 kPa, an arterial carbon dioxide tension (P_{a,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. Arterial glucose levels and haematocrit were determined at the beginning and the end of the experiments, and were found to have remained within normal values. Supplemental oxygen or bicarbonate was not given during the observation time.

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. Respiratory pressure was measured by connecting an outlet of the extratracheal tube to a Statham PM 131 TC pressure transducer and this value was used as a measure of transrespiratory pressure (P), since intratracheal pressure was equal to atmospheric pressure because of the thoracotomy. Airflow (F) was measured with a heated Fleisch No. 1 pneumotachograph connected to a Statham PM 15 E pressure transducer. F and P signals were sent to an AP 200 Pulmonary Computer (ConMeTech AB, Uppsala, Sweden) for on-line calculations of resistance (R) and C_{dyn}, see below for further details. 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.

Experimental procedures

A skin test using a standardized extract of *A. suum* in a tenfold dilution series was performed to classify the degree of sensitivity to the allergen. The allergen was given intradermally, at a volume of 50 µL per site one hour prior to the aerosol challenge. The end-point of titration was determined as the lowest concentration giving a dark reddening exceeding 2 mm after 10 min. Pigs responding only to undiluted extract were classified as Class 1-responders, those responding to a tenfold dilution were classified as Class 2, those responding to a 100 fold dilution Class 3, *etc.* Only those pigs that were identified as Class 2-responders or more were included in the study.

Eight of the sensitized and five of the nonsensitized pigs were given a bolus dose of metyrapone (Sigma, St. Louis, MO, USA) of 25 mg·kg^{-1} 2 h before the allergen challenge (1 p.m.), followed by a continuous *i.v.* infusion (10 mg·kg^{-1}·h^{-1}). Challenge with the nebulized allergen was performed 1.5 h after the end of the surgical preparation. The allergen consisted of an extract of *A. suum*, with a protein concentration of 7 mg·mL^{-1} in a volume of 2.0 mL of saline. The aerosol was generated using an ultrasonic nebulizer (NB 108, Engström Medical, Stockholm, Sweden) and was delivered over 5 min *via* the tracheal tube to the lower airways. Arterial blood was drawn 15 min and 2 min before, and 15 min, 1, 2, 4, 6 and 8 h after the allergen challenge. At the end of the experiments the animals were killed with an overdose of pentobarbi-tone.

Sample processing and analysis

Blood was gently mixed with ethylenediamine tetraacetic acid (EDTA) to a final concentration of 10 mM. The blood samples were kept on ice for a maximum of 30 min, followed by centrifugation at 4°C, 680×g for 10 min. Plasma was collected and stored at -70°C until analysis of cortisol, adrenaline and noradrenaline.

Plasma concentrations of cortisol were determined using a radio-immunoassay (Orion Diagnostica AB, Trosa, Sweden), with a detection limit of 3–5 nM.

Catecholamines (adrenaline and noradrenaline) were determined after alumina extraction by cation-exchange high-performance liquid chromatography (HPLC) with electrochemical detection, according to HJEMDAHL [11].

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. Area under the curve for VR during the acute reaction ($AUC_{0–2\ h}$) was calculated by using 0% as a baseline. When calculating the AUC during the late reaction ($AUC_{2–8\ h}$) the baseline was set to the maximal value of VR, seen between the acute and late fall in VR. The eventual vasoconstriction between the acute and late reaction was not included in the AUCs.

R and C_{dyn} were calculated as described by MEAD [12]. *R* was calculated using the formula $R=(P_1-P_2)/(F_1-F_2)$ at the 50% isovolumetric level. The resistance of the pneumotachograph (4.0 $cmH_2O \cdot L^{-1} \cdot s$) was subtracted from the calculated *R* to achieve total lung resistance (R_L). C_{dyn} was calculated as tidal volume/P_{max}.

Data are presented as mean±SEM. Statistical evaluations were performed using the Quade test for nonparametric two-way analysis of variance and multiple comparisons on ranks of several related samples [13], Mann-Whitney U-test, Wilcoxon signed ranks test, and Spearman's rank correlation using Statistica (Statsoft) and Kruskal-Wallis was performed using InStat (GraphPad Software) on an Apple Macintosh computer.

Results

Classification of sensitized pigs by skin test

After intradermal injections of the *A. suum* allergen, an immediate light red flare reaction, lasting for 1–3 min, followed by a darker reddening that started to develop after about 3 min could be seen in sensitized pigs. Unsensitized pigs did not respond to the allergen.

The classification of the sensitized animals according to allergen reactivity in the skin showed a majority, 10, of the pigs to be Class 3-responders. Four pigs were Class 4, and one pig was Class 2. Two of the 17 pigs were Class 1 and were excluded from the study. Acute airway reactivity (fall in C_{dyn}) correlated better with titration of the *A. suum* allergen (r=-0.51, p<0.05, Spearman's rank correlation) than with the area of the skin reaction of a standard concentration of the allergen (tenfold dilution, r=-0.43, p>0.10, Spearman's rank correlation).

Non-metyrapone-treated pigs did not show late-phase skin reactions, whereas all except one in the metyrapone-treated group exhibited persistent late-phase skin reactions to the two highest concentrations of allergen. This late-phase response consisted of a pale red reaction, with a slightly larger area than the acute response, and lasted for at least 8 h.

Plasma cortisol, adrenaline and noradrenaline

The plasma cortisol level in sensitized pigs at 9 a.m., after premedication with ketamine and in conjunction with the induction of anaesthesia, was within the normal range (151±28 nM, n=6). In sensitized pigs not treated with metyrapone, there was a continuous increase in plasma cortisol resulting in midday levels (after the surgical preparation and 15 min before the allergen challenge) three times higher than at 9 a.m. (table 1, fig. 1). Metyrapone treatment inhibited this increase, resulting in lower midday cortisol levels (table 1, fig. 1), *i.e.* about 2 h after the bolus injection of metyrapone. Already 15 min after the bolus dose, a decrease in circulating cortisol levels to 60% was noted. At 8 h, no change in cortisol levels was seen in the non-metyrapone-treated groups compared to prechallenge levels, whilst in sensitized metyrapone-treated pigs a mean further reduction of 43 and 54% could be detected in sensitized and non-sensitized pigs, respectively (fig. 1).

In two pigs, plasma adrenaline levels were measured every 15 min after induction of anaesthesia. Peak plasma adrenaline (about 1.5 nM) was achieved within 15 min after induction of anaesthesia, but returned to low levels within 30 min. Fifteen minutes before allergen challenge, plasma adrenaline levels were still low in non-metyrapone-treated and nonsensitized, metyrapone-treated pigs but had increased in sensitized, metyrapone-treated pigs (table

Table 1. – Prechallenge levels (15 min before allergen challenge) of physiological parameters and plasma mediators in three groups of pigs

	Sensitized, non-metyrapone treated (n=7)	Sensitized, metyrapone treated (n=8)	Nonsensitized, metyrapone treated (n=5)
Cortisol nM	455±37	40.1±3.8**	33.3±2.3**
Adrenaline nM	0.34±0.11	3.2±1.3*	0.45±0.23
Noradrenaline nM	0.73±0.20	0.60±0.13	0.31±0.07
MAP mmHg	134±3	113±6*	119±3
Heart rate b.p.m.	146±15	135±9	137±14
R_L $cmH_2O \cdot L^{-1} \cdot s$	4.1±0.4	3.7±0.7	6.4±1.4
C_{dyn} $mL \cdot cmH_2O^{-1}$	36±4	34±2	30±5
Bronchial VR $mmHg \cdot mL^{-1} \cdot min$	23±9	9.3±0.6	8.7±2.3
P_{a,O_2} kPa	13.2±0.7	14.3±0.7	13.7±0.6
P_{a,CO_2} kPa	5.6±0.3	5.0±0.2	5.1±0.1
pH	7.42±0.02	7.46±0.02	7.46±0.004

Data are presented as mean±SEM. MAP: mean arterial pressure; b.p.m.: $beats \cdot min^{-1}$; R_L: total lung resistance; Cdyn: dynamic compliance; VR: vascular resistance; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension. *: p<0.05; **: p<0.01: compared to sensitized, non-metyrapone pigs (Kruskal-Wallis).

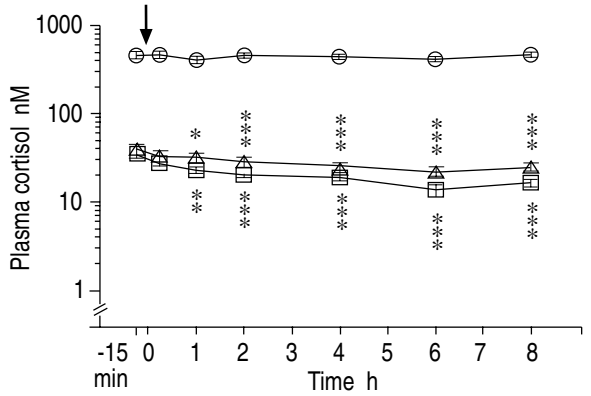

Fig. 1. – Plasma cortisol levels (nM) mean and SEM at various times before and after allergen challenge. —○— : sensitized, non-metyrapone-treated (n=6); —Δ— : sensitized, metyrapone-treated (n=8); —❑— : nonsensitized, metyrapone-treated (n=5). Arrow indicates allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test). Note that vertical axis is cut-off from zero.

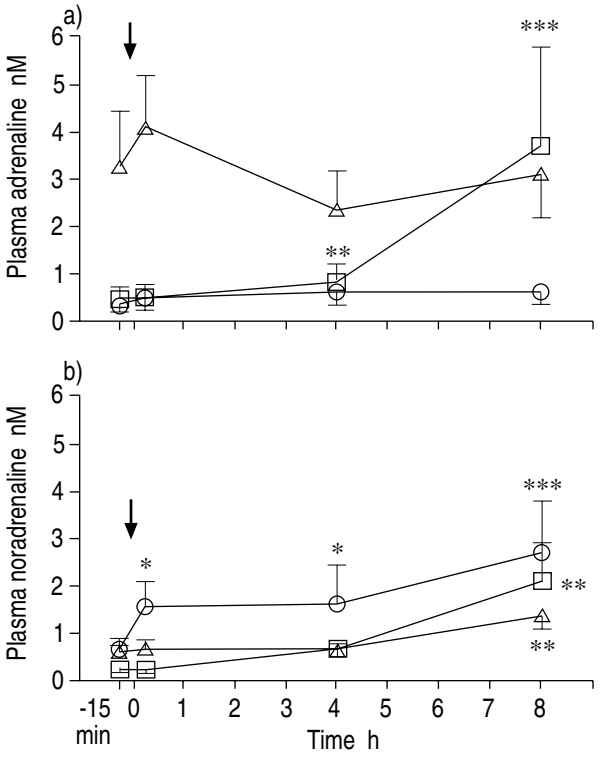

Fig. 2. – Plasma levels of: a) adrenaline; and b) noradrenaline before and after allergen challenge. Values are presented as nM mean and SEM. —○—: sensitized, non-metyrapone-treated (n=6); —Δ—: sensitized, metyrapone-treated (n=8); —❑— : nonsensitized, metyrapone-treated (n=5). Arrow indicates allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

1, fig. 2a). Fifteen minutes after the start of allergen challenge, plasma adrenaline had increased to 111±72% (p<0.05, Wilcoxon signed ranks test) of the prechallenge level in the sensitized, metyrapone-treated pigs. The levels of adrenaline did not change acutely in sensitized, non-metyrapone-treated pigs, however. Adrenaline levels returned to baseline levels 4 h after allergen challenge and remained unaltered during the rest of the observation period in the two sensitized groups. In the nonsensitized, metyrapone-treated group, adrenaline levels were unaltered at 4 h but at 8 h had increased about 8 times compared to baseline, *i.e.* to levels comparable with those in the sensitized, metyrapone-treated group.

The levels of noradrenaline 15 min before allergen challenge were low in all pigs (table 1, fig. 2b). In the sensitized, non-metyrapone-treated group, plasma noradrenaline showed a significant increase of 135±54% 15 min after the allergen challenge (p<0.05; Wilcoxon signed ranks test). Noradrenaline levels tended to increase 15 min after allergen challenge in sensitized pigs treated with metyrapone (a rise of 31±15%; p<0.10, Wilcoxon signed ranks test). A further rise in noradrenaline levels was seen at 8 h in all pigs.

Mean arterial pressure and heart rate

The MAP was 16% lower in sensitized, metyrapone-treated pigs than in sensitized, non-metyrapone-treated pigs 15 min before allergen challenge (table 1). There was no difference in heart rate 15 min before allergen challenge in the three groups (table 1).

The allergen challenge caused an acute increase in MAP and heart rate that peaked at 10±1 min and returned to baseline after 19±2 min in both sensitized groups. The MAP increased to 148±6 and 120±10 mmHg in sensitized, non-metyrapone-treated and sensitized, metyrapone-treated pigs, respectively, and the heart rate increased to 177±17 and 159±15 beats·min^{-1}, respectively. During the course of the day, there was a steady decrease in MAP, resulting in a significantly lower blood pressure at 8 h compared to prechallenge value in all pigs (94±8 and 77±3 mmHg for sensitized, non-metyrapone-treated and sensitized, metyrapone-treated pigs, respectively; p<0.05, Quade test). After the acute response had resolved, the heart rate was not further changed in any of the groups during the rest of the observation period. Except during the acute-phase reaction, no difference in either MAP or heart rate was found between sensitized, metyrapone-treated and nonsensitized, metyrapone-treated pigs.

Total lung resistance and dynamic compliance

Basal R_L and C_{dyn} (table 1) were not affected by metyrapone-pretreatment. Challenge of sensitized pigs with *A. suum* aerosol resulted in a significant increase in R_L of 85±27% in the non-metyrapone-treated group (time to reach maximum 10±2 min) (fig. 3a) and 59±13% in the pigs treated with metyrapone (same time pattern, fig. 3b). The magnitude of the maximal acute airways obstruction was not significantly different between the two groups (Mann-Whitney U-test). Unsensitized pigs did not react when challenged with *A. suum* allergen (fig. 3c). The acute increase in R_L resolved within 60±8 min in the non-metyrapone-treated group, but was more prolonged in the metyrapone-treated group, lasting 94± 10 min (p<0.05, Mann-Whitney U-test). A late increase in R_L, starting at 3.7±0.2 h was seen only in the sensitized, metyrapone-treated group. At 8 h, R_L had increased by 48±8% above baseline, without any sign of having reached a plateau. The two sensitized groups were significantly different from each other at 7 and 8 h after challenge (p<0.05, Mann-Whitney U-test).

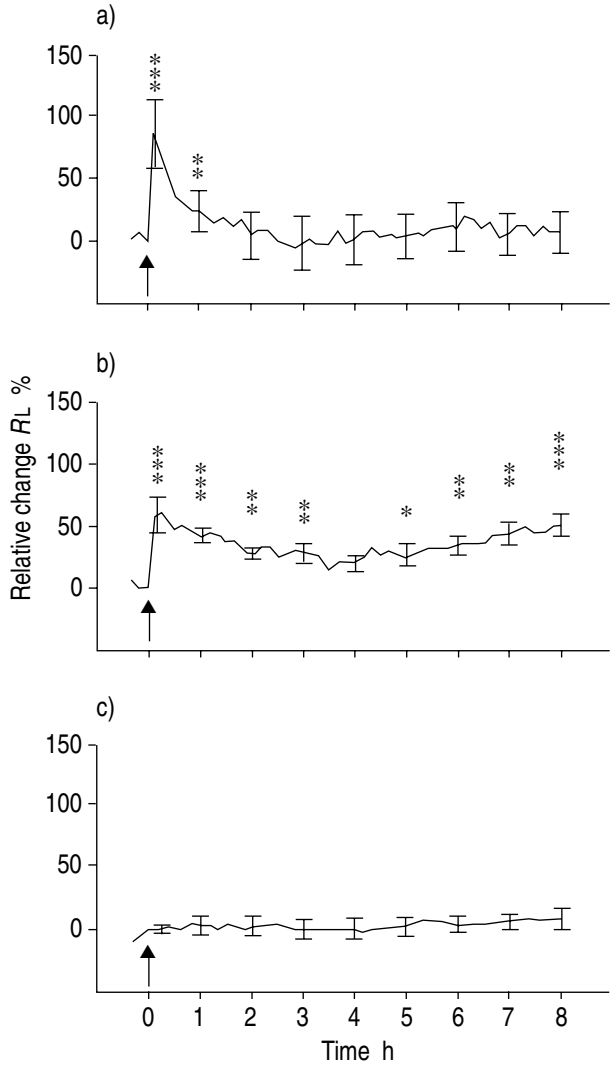

Fig. 3. – Relative change from baseline in total lung resistance (R_L) after allergen challenge in: a) sensitized, non-metyrapone-treated; b) sensitized, metyrapone-treated; and c) nonsensitized, metyrapone-treated pigs. Values are presented as mean±SEM (n=5–8). Arrow indicates allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

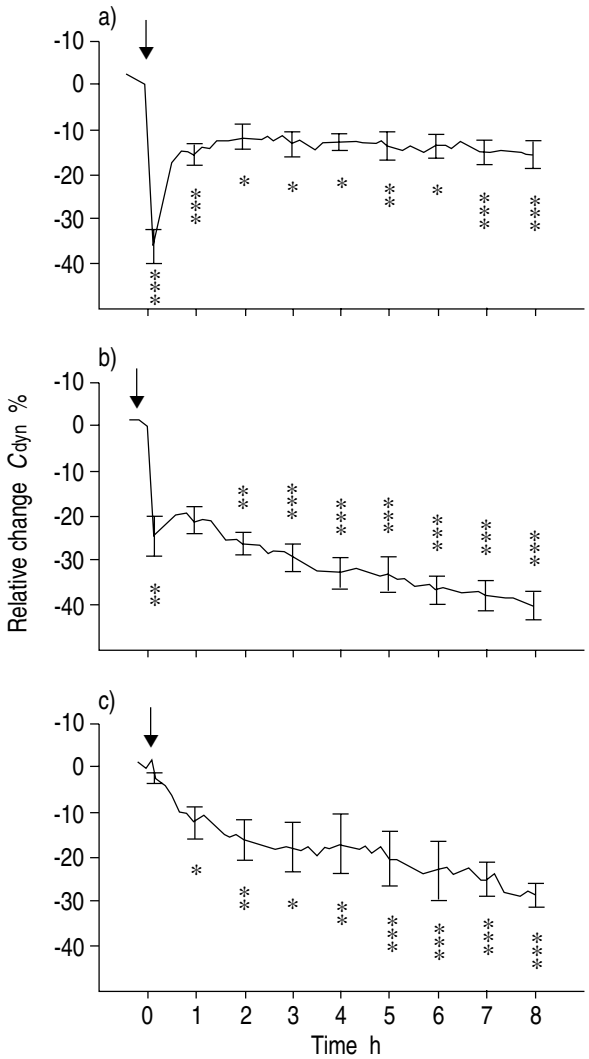

Fig. 4. – Relative change from baseline in dynamic compliance (C_{dyn}) after allergen challenge in: a) sensitized, non-metyrapone-treated; b) sensitized, metyrapone-treated; and c) nonsensitized, metyrapone-treated pigs. Values are presented as mean±SEM (n=5–8). Arrow indicates allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

Allergen challenge in sensitized pigs resulted in an acute decrease in C_{dyn} that was similar in the two sensitized groups, consisting of a decrease by 36±4% in non-metyrapone-treated pigs and 25±4% in metyrapone-treated pigs (fig. 4 a and b). Time to reach minimum did not differ significantly in the two sensitized groups (10±1 and 16±7 min for non-metyrapone-treated pigs and metyrapone-treated pigs, respectively). No acute effect on C_{dyn} was seen in nonsensitized, metyrapone-treated pigs. Relative C_{dyn} values in sensitized, metyrapone-treated pigs were significantly different from those in sensitized, non-metyrapone-treated pigs between 2–8 h after challenge, and significantly different (p<0.05) from those in nonsensitized, metyrapone-treated pigs at 7 and 8 h after challenge. A decrease in C_{dyn} by 14±4% 8 h after allergen challenge was seen in nonsensitized, non-metyrapone-treated pigs (n=3, not shown). Metyrapone treatment by itself increased this decline in C_{dyn} to 29±2% after allergen challenge (fig. 4c).

P_{a,O_2}, P_{a,CO_2}, pH and base excess

Metyrapone treatment did not affect basal arterial blood gas levels, pH or base excess (table 1). After allergen challenge, there was an acute decrease in P_{a,O_2} and pH in all sensitized pigs, and these responses resolved at 2–4 h in non-metyrapone-treated pigs (figs 5a and b and 6a and b). In sensitized, metyrapone-treated pigs, P_{a,O_2} and pH did not return to baseline, and for pH even started to decrease again. The allergen challenge caused an acute rise in P_{a,CO_2} in sensitized pigs, which resolved completely at 2 h in non-metyrapone-treated pigs (fig. 7a). In sensitized, metyrapone-treated pigs, P_{a,CO_2} did not return to baseline before starting a second increase (fig. 7b). A decrease in pH (fig. 6a and b) was always accompanied both by an increase in P_{a,CO_2} (fig. 7a and b) and a decrease in base excess (not shown). P_{a,O_2}, P_{a,CO_2}, pH and base excess were not affected by allergen challenge in nonsensitized, metyrapone-treated animals (figs 5c, 6c and 7c).

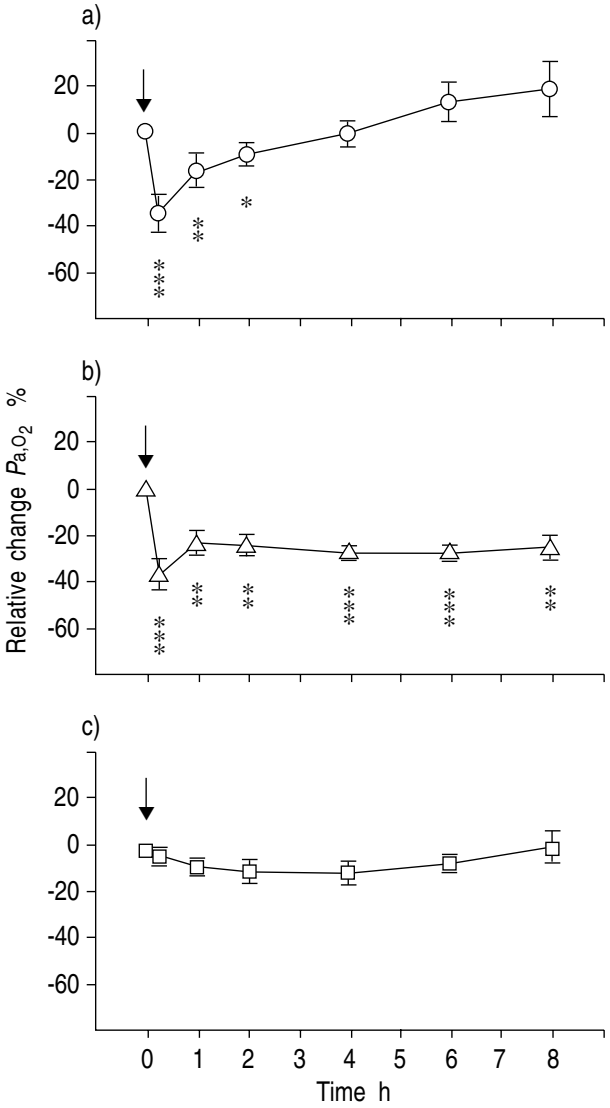

Fig. 5. – Relative change from baseline in arterial oxygen tension (P_{a,O_2}) after allergen challenge in: a) sensitized, non-metyrapone (○); b) sensitized, metyrapone-treated (Δ); and c) nonsensitized, metyrapone-treated pigs (❑). Values are presented as mean±SEM (n=5–8). Arrows indicate allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

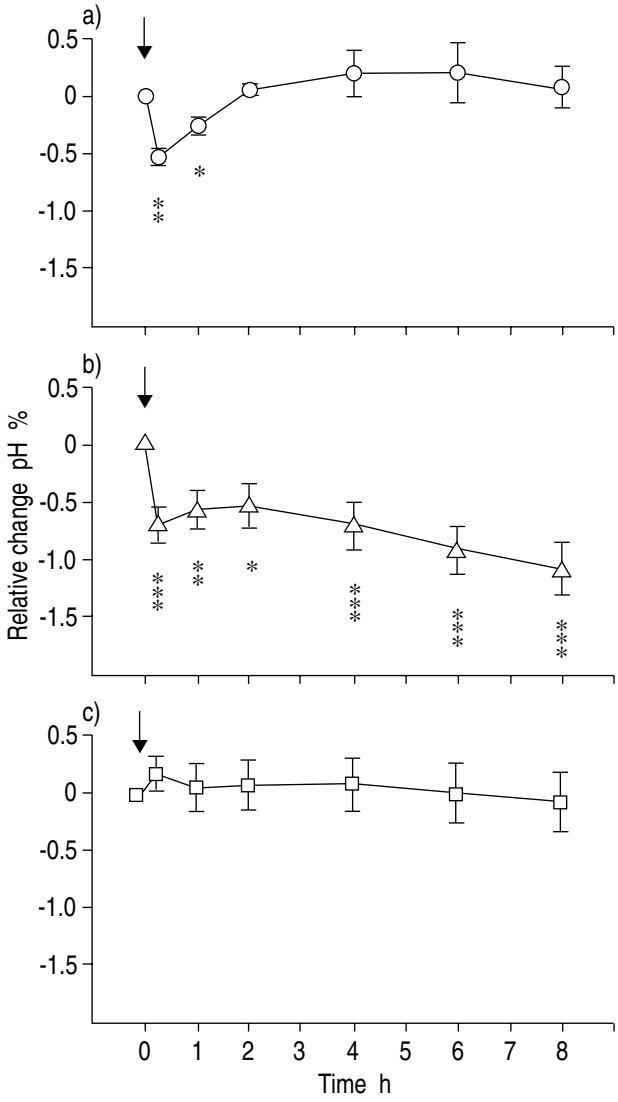

Fig. 6. – Relative change from baseline in arterial pH after allergen challenge in: a) sensitized, non-metyrapone-treated (○); b) sensitized, metyrapone-treated (Δ); and c) nonsensitized, metyrapone-treated pigs (❑). Values are presented as mean±SEM (n=5–8). Arrows indicate allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

Bronchial vascular resistance

Basal bronchial VR was not significantly affected by metyrapone treatment (table 1). Allergen challenge caused a similar acute vasodilatory response in the bronchial circulation in both sensitized groups, measured as a decrease in calculated VR (fig. 8a and b). The peak was seen at 16±2 min (p<0.05 compared to nonsensitized pigs, Mann-Whitney U-test) and the duration, which was not significantly different in the two groups, was 70±8 and 56±10 min in non-metyrapone-treated and metyrapone-treated pigs, respectively. $AUC_{0–2\ h}$ was not different in the two sensitized groups (-32±12 and -30±16%·h^{-1} in non-metyrapone-treated and metyrapone-treated pigs, respectively). In both groups, there was a significant (p<0.05) difference compared to the nonsensitized group (10±11%·h). In both sensitized groups, a consistent late-phase vasodilatory response was noted, but with variable time to peak effect in different pigs. $AUC_{2–8\ h}$ was not different in the two sensitized groups (-241±80 and -119±54%·h in non-metyrapone-treated and metyrapone-treated pigs, respectively). The $AUC_{2–8\ h}$ for sensitized, non-metyrapone pigs (16±41%·h) was significantly different (p<0.01) from the nonsensitized group, whilst $AUC_{2–8\ h}$ for sensitized, metyrapone-treated pigs only tended to be different from the nonsensitized group (p=0.08). When calculating the group mean, the peak late-phase decrease in VR was found to occur earlier for metyrapone-treated pigs than for non-metyrapone-treated pigs (5.0±0.4 h compared to 6.9±0.6 h; p<0.01). In nonsensitized animals, no consistent changes in VR were noted during 8 h after allergen challenge (fig. 8c).

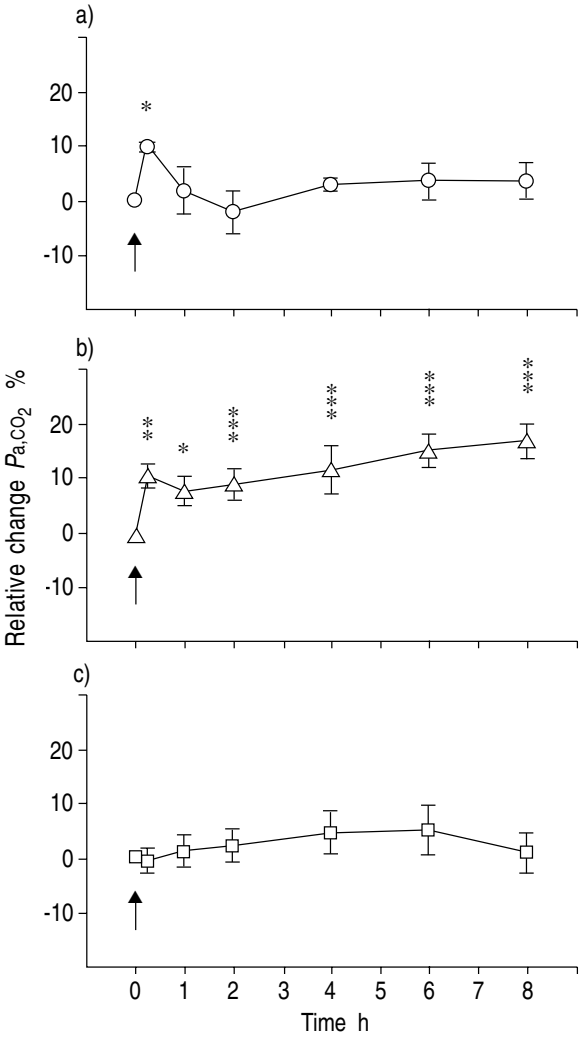

Fig. 7. – Relative change from baseline in arterial carbon dioxide tension (P_{a,CO_2}) after allergen challenge in: a) sensitized, non-metyrapone-treated (○); b) sensitized, metyrapone-treated (Δ); and c) nonsensitized, metyrapone-treated pigs (□). Values are presented as mean±SEM (n=5–8). Arrows indicate allergen challenge. *: p<0.05; **: p<0.01; ***: p<0.001, compared to baseline (Quade test).

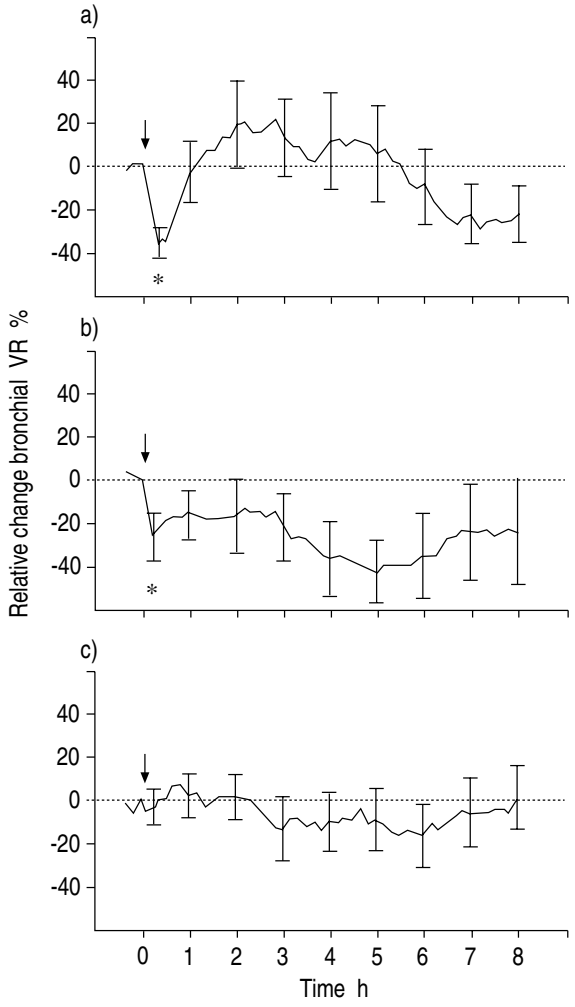

Fig. 8. – Relative change from baseline in vascular resistance (VR) in the bronchial artery after allergen challenge in: a) sensitized, non-metyrapone-treated; b) sensitized, metyrapone-treated; and c) nonsensitized, metyrapone-treated pigs. Values are presented as mean±SEM (n=5–7). Arrows indicate allergen challenge. *: p<0.05, compared to nonsensitized pigs (Mann-Whitney U-test).

Discussion

In this study, we have described the late-phase response in actively sensitized pigs in terms of pulmonary and cardiovascular parameters and their relation to plasma cortisol and catecholamine levels.

Late-phase reactions in response to allergen challenge in the lower airways do not appear in all individuals, as shown by studies both in humans [14] and animals [15], and the reason for this is still unclear. The mechanisms involved in the late-phase reactions are also thought to play a part in the development of chronic inflammation in bronchial asthma [16], and it is, therefore, of value to characterize more closely the late-phase reaction. Both the likelihood of developing a late asthmatic reaction in humans and the severity and time course of the late response are dependent on the time of day at which allergen challenge is performed, whereas the acute reaction seems to be independent of the time of challenge [2]. The timing of administration of synthetic glucocorticoids is also critical to achieve maximum effect on nocturnal asthma, which may be a kind of late-phase reaction [2, 17].

One of the main candidates for orchestrating the inflammatory response is cortisol, which shows a marked circadian variation, with the lowest plasma levels found around midnight [6]. Furthermore, nocturnal asthma can be inhibited by intravenous administration of cortisol [3]. Experiments using metyrapone have shown that decreased endogenous cortisol levels are of critical importance for the development of late-phase reactions in dogs [18]. Metyrapone inhibits the 11β-hydroxylase system and, thus, blocks the final stage in the biosynthesis of cortisol from 11-deoxycortisol [4]. Lowered plasma levels of cortisol will lead to an increase of the production of adrenocorticotrophic hormone (ACTH), which in turn increases the levels of the cortisol-precursor 11-deoxycortisol. This precursor does not possess any glucocorticoid effect, however. A reduction in aldosterone synthesis is also seen [5], which results in an increased

production of 11-deoxycorticosterone, and this intermediary substance also possesses mineralocorticoid effects like aldosterone.

The effect of metyrapone on cortisol excretion from the adrenals is rapid, and maximal effect was obtained 10 min after *i.v.* administration in dogs [4]. In this species, the cortisol suppressive effect of a single dose of metyrapone (75 $mg \cdot kg^{-1}$) lasted approximately 3 h [19]. We therefore chose to administer metyrapone continuously by intravenous infusion in the pig. This procedure resulted in a progressive decrease in cortisol levels over the day, similar to normal circadian variation [6]. Daytime plasma cortisol levels in normal conscious pigs range 75–140 nM [20], with the highest level in the morning [21], as in humans. This suggests that the cortisol levels at 8 h after allergen challenge in metyrapone-treated pigs should be close to normal midnight values in the pig. The cortisol levels are regulated by ACTH, and an intravenous injection of ACTH in pigs increased the plasma cortisol levels to approximately 330 nM 1–2 h after the injection [21]. Trauma, including surgery, makes the ACTH levels and subsequently cortisol levels rise [22], independent of anaesthesia [22, 23]. We have previously reported postsurgical cortisol levels in non-pathogen-free pigs of about 240 nM [24], which is higher than in unrestrained, conscious pigs [20]. Late-phase reactions in response to allergen challenge were found in non-pathogen-free pigs, in spite of relatively high cortisol levels [10]. However, when, as in the present study, pathogen-free pigs were used, no late-phase bronchoconstrictor response could be seen. This could be due to higher postsurgical cortisol levels in the pathogen-free breed compared to normal domestic pigs, or possibly due to the total absence of pre-existing inflammation in the airways in the barrier-bred animals.

After metyrapone administration, an increase in adrenal vein excretion of catecholamines can be seen, starting at about 5 min and lasting at least 2 h, according to CHART and SHEPPARD [4]. We could see an increase in circulating adrenaline levels within 2 h after the metyrapone bolus injection in sensitized pigs, but only after 10 h in nonsensitized pigs. The reason for this difference remains to be elucidated. An increase in noradrenaline from normal values [25] was seen acutely after allergen challenge in non-metyrapone pigs only. It has been shown that cortisol inhibits catecholamine uptake-2 [26], monoamine oxidase [27] and catechol-*O*-methyl transferase [28], all pathways for clearance of released noradrenaline. The inhibition of these pathways in pigs with high cortisol levels could possibly explain the long-lasting increase of this amine in the non-metyrapone-treated group after allergen provocation. The increase in plasma noradrenaline seen in all groups towards the end of the experiment, is probably related to the reduction in MAP causing sympathetic activation. Taken together, these results show that adrenaline levels did not correlate inversely with the development of late-phase reaction, as suggested by BARNES *et al.* [6] and BATES *et al.* [7] for nocturnal asthma. Instead, plasma cortisol levels seem to be the main factor determining the inflammatory outcome of allergen challenge, in line with results from studies on the development of late-phase reactions in humans [2, 29].

Metyrapone treatment in the doses given to the pig caused a gradual decrease in MAP throughout the experiment. Similarly, in Addison's disease, hypotension, probably due to low cortisol levels, is a common clinical sign [30]. The decrease in MAP in the pigs could not be counteracted by giving supplemental isotonic fluid intravenously, and since arterial haematocrit was not changed during the experiment, it is suggested that the reduction in MAP was not caused by fluid loss. Furthermore, metyrapone did not seem to exert a general cardiac depressive effect, since the heart rate was not significantly affected. The exact mechanism for the slowly developing hypotension caused by metyrapone in the pig thus remains unclear, but the phenomenon can be avoided by choosing a lower dose of metyrapone that does not reduce MAP, but still permits late-phase reactions to occur (unpublished results). In this study, a high dose of metyrapone was chosen to induce clear-cut effects on cortisol synthesis.

The sensitization procedure was performed in a way described earlier, probably yielding reaginic antibodies of the immunoglobulin E (IgE) isotype [9]. Determination of the sensitivity of the skin was performed by titration of the *A. suum* allergen as described in humans by WEEKE and POULSEN [31], and in the pig, airway reactivity correlated better with the end-point of the titration of allergen in the skin than to the area of the skin reaction. The pig skin, which resembles human skin, is suitable for allergen skin testing since the response is visible without the use of Evans blue. The result of the sensitization procedure was satisfactory, since only 2 out of 17 pigs were excluded from the study due to poor response to allergen challenge in the skin. Furthermore, a late-phase reaction in the skin resembling late skin reactions after metyrapone treatment in humans [29] was found.

During the acute bronchoconstrictor response a prominent decrease in P_{a,O_2} was seen, indicating that arterial oxygen levels could be as sensitive as pulmonary mechanics [32] as indicators of bronchoconstriction. A decrease in blood pH was seen both during the acute and the late reaction in the airways, and since this decrease was always accompanied both by an increase in P_{a,CO_2} and a decrease in base excess, we suggest that the acidosis consisted both of a respiratory and a metabolic component. The acute bronchoconstrictor response in the pig lung after allergen challenge is to a large extent mediated by histamine, since it is largely reduced by a combination of H_1- and H_2-histamine receptor antagonists [33], but the mediator mechanism in the late-phase airways obstruction remains to be determined.

To make it possible to study changes in C_{dyn}, a positive-end expiratory pressure was avoided in this study. A slight decrease in C_{dyn} was seen during primarily the first hour of the experiment in non-metyrapone-treated pigs, even in animals not receiving allergen. This is suggested to be caused by atelectasis formation. The more profound decrease seen in non-sensitized, metyrapone-treated pigs, which was continuous over 8 h, may

be caused by abdominal distension due to generalized oedema [34]. Alveolar oedema is unlikely, since Pa,CO_2 was not changed in these animals.

Late changes in bronchial vascular resistance after allergen challenge have previously been reported in sheep [35] and pigs [10]. The acute vasodilatation in the bronchi seems to be mediated, at least to some extent, by locally released histamine, both in the pig [33, 36] and the sheep [37], but the origin of the late reaction has not yet been determined. The late vasodilator response was present in pigs without late-phase airways obstruction as well as in pigs with airways obstruction, which is in contrast to findings in the sheep [35]. Furthermore, the time course of late bronchial blood flow changes varied in different pigs, suggesting that the late-phase airways obstruction and bronchial vasodilator reactions are independent of each other, at least in the pig. It can be concluded that the late vasodilator response is not affected to the same extent as the late airways obstruction by high cortisol levels.

In this study, we have shown the importance of endogenous cortisol levels for the control of late-phase reactions to allergen challenge in the lower airways of the pig, as judged by changes in pulmonary airway mechanics, blood gas partial pressures and pH. We found that high endogenous cortisol levels can inhibit the development of late-phase reactions in the lung, whereas increased adrenaline levels do not seem to influence these reactions. However, the bronchial circulation showed a late vasodilator response to allergen challenge despite high cortisol levels and absence of airways obstruction.

Acknowledgements: The authors thank M. Stensdotter and C. Nihlén for expert technical and laboratory assistance.

References

1. Booij-Noord H, Orie NGM, de Vries K. Immediate and late obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J Allergy Clin Immunol* 1971; 48: 344–354.
2. Mohiuddin AA, Martin RJ. Circadian basis of the late asthmatic response. *Am Rev Respir Dis* 1990; 142: 1153–1157.
3. Beam WR, Ballard RD, Martin RJ. Spectrum of corticosteroid sensitivity in nocturnal asthma. *Am Rev Respir Dis* 1992; 145: 1082–1086.
4. Chart JJ, Sheppard HJ. Pharmacology and biochemistry of some amphenone analogues and other adrenal cortical inhibitors. *J Med Pharm Chem* 1959; 1: 407–441.
5. Kahnt FW, Neher R. On the specific inhibition of adrenal steroid biosynthesis. *Experientia* 1962; 18: 499–501.
6. Barnes P, FitzGerald G, Brown M, *et al.* Nocturnal asthma and changes in circulating epinephrine, histamine, and cortisol. *N Engl J Med* 1980; 303: 263–267.
7. Bates ME, Clayton M, Calhoun W, *et al.* Relationship of plasma epinephrine and circulating eosinophils to nocturnal asthma. *Am J Respir Crit Care Med* 1994; 149: 667–672.
8. Smith H. Animal models of asthma. *Pulm Pharmacol* 1989; 2: 59–74.
9. Alving K. Airways vasodilatation in the immediate allergic reaction: involvement of inflammatory mediators and sensory nerves. *Acta Physiol Scand* 1991; 141 (Suppl. 597): 1–64.
10. Alving K, Matran R, Fornhem C, *et al.* Late-phase bronchial and vascular responses to allergen in actively-sensitized pigs. *Acta Physiol Scand* 1991; 143: 137–138.
11. Hjemdahl P. Catecholamine measurements in plasma by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol* 1987; 142: 521–534.
12. Mead J. Mechanical properties of lungs. *Physiol Rev* 1961; 41: 281–330.
13. Theodorsson-Norheim E. Friedman and Quade test: basic computer program to perform nonparametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput Biol Med* 1987; 17: 85–99.
14. Dahl R. Early- and late-phase reactions in the bronchi and the nose. *In*: Mygind N, Pipkorn U, Dahl R, eds. Rhinitis and Asthma: Similarities and Differences. Copenhagen, Munksgaard, 1990; pp. 203–212.
15. Abraham WM. Pharmacology of allergen-induced early and late airway responses and antigen-induced airway hyperresponsiveness in allergic sheep. *Pulm Pharmacol* 1989; 2: 33–40.
16. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987; 136: 740–751.
17. Beam WR, Weiner DE, Martin RJ. Timing of prednisone and alterations of airways inflammation in nocturnal asthma. *Am Rev Respir Dis* 1992; 146: 1524–1530.
18. Sasaki H, Yanai M, Shimura S, *et al.* Late asthmatic response to *Ascaris* antigen challenge in dogs treated with metyrapone. *Am Rev Respir Dis* 1987; 136: 1459–1465.
19. Jenkins JS, Meakin JW, Nelson DH, *et al.* Inhibition of adrenal steroid 11-oxygenation in the dog. *Science* 1958; 128: 478–480.
20. Stuart G, Spencer G, Hallett KG. Hormone and metabolite changes with stress in pigs. *In*: Tumbleson ME, ed. Swine in Biomedical Research. Vol. 1–3. New York, Plenum Press, 1986; pp. 159–165.
21. Becker BA, Ford JJ, Nienaber JA, *et al.* Endocrine and behavior changes associated with intensive housing system for swine. *In*: Tumbleson ME, ed. Swine in Biomedical Research. Vol. 1–3. New York, Plenum Press, 1986; pp. 173–189.
22. Hume DM, Bell CC, Bartter F. Direct measurement of adrenal secretion during operative trauma and convalescence. *Surgery* 1962; 52: 178–187.
23. Hjortsø NC, Christensen NJ, Andersen T, *et al.* Effects of the extradural administration of local anaesthetic agents and morphine on urinary excretion of cortisol, catecholamines and nitrogen following abdominal surgery. *Br J Anaesth* 1985; 57: 400–406.
24. Alving K, Matran R, Lundberg JM. Capsaicin-induced local effector responses, autonomic reflexes and sensory neuropeptide depletion in the pig. *Naunyn-Schmiedeberg's Arch Pharmacol* 1991; 343: 37–45.
25. Hannon JP. Hemodynamic characteristics of the conscious resting pig: a brief review. *In*: Tumbleson ME, ed. Swine in Biomedical Research. Vol. 1–3. New York, Plenum Press, 1986; pp. 1341–1352.
26. Iversen LL, Salt PJ. Inhibition of catecholamine uptake 2 by steroids in the isolated rat heart. *Br J Pharmacol* 1970; 40: 528–530.
27. Parvez H, Parvez S. Control of catecholamine release and degradation by the glucocorticoids. *Experientia* 1972; 28: 1330–1332.

28. Kalsner S. Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circ Res* 1969; 24: 383–395.
29. Herrscher RF, Kasper C, Sullivan TJ. Endogenous cortisol regulates immunoglobulin E-dependent late phase reactions. *J Clin Invest* 1992; 90: 596–603.
30. Williams GH, Dluhy RG. Diseases of the adrenal cortex. *In*: Wilson JD, Braunwald E, Isselbacher KJ, eds. Harrison's Principles of Internal Medicine. 12th edn. Vol. 2. New York, McGraw-Hill, 1991; pp. 1713–1735.
31. Weeke B, Poulsen LK. Diagnostic tests for allergy. *In*: Holgate ST, eds. Allergy. London, Gower Medical Publishing, 1993; pp. 11.1–11.14.
32. Holmgren D, Sixt R. Transcutaneous and arterial blood gas monitoring during acute asthmatic symptoms in older children. *Pediatr Pulmonol* 1992; 14: 80–84.
33. Alving K, Matran R, Lacroix JS, *et al.* Capsaicin and histamine antagonist-sensitive mechanisms in the immediate allergic reaction of pig airways. *Acta Physiol Scand* 1990; 138: 49–60.
34. Mutoh T, Lamm WJ, Embree LJ, *et al*. Volume infusion produces abdominal distension, lung compression, and chest wall stiffening in pigs. *J Appl Physiol* 1992; 72: 575–582.
35. Long WM, Yerger LD, Abraham WM, *et al.* Late-phase bronchial vascular responses in allergic sheep. *J Appl Physiol* 1990; 69: 584–590.
36. Alving K, Matran R, Lacroix JS, *et al.* Allergen challenge induces vasodilatation in pig bronchial circulation *via* a capsaicin-sensitive mechanism. *Acta Physiol Scand* 1988; 134: 571–572.
37. Long WM, Yerger LD, Martinez H, *et al.* Modification of bronchial blood flow during allergic airway responses. *J Appl Physiol* 1988; 65: 272–282.