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Airway Reactivity to Inhaled Spasmogens 18–24 h after Antigen-challenge in Sensitized Anaesthetized Guinea-pigs

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Abstract

The anaesthetized allergic guinea-pig was used to assess changes in airway reactivity to four different inhaled spasmogens: methacholine, 5-hydroxytryptamine (5-HT), histamine and the thromboxane A_2 mimetic, 9,11-dideoxy-9α,11α-methano-epoxy-$PGF_{2\alpha}$ (U-46619). Reactivity was determined 18 to 24 h after challenge of ovalbumin-sensitized guinea-pigs with inhaled ovalbumin. This time coincides with the appearance of a late-phase bronchoconstriction in these animals. Sensitivity to the spasmogen was assessed from the concentration–response curve for the increase in pulmonary inflation pressure (PIP) in ovalbumin- and saline-challenged sensitized animals.

When methacholine, 5-HT or histamine were the spasmogens there was no hyper-reactivity. The geometric mean EC50 values (i.e. the concentrations inducing half the maximum effect) obtained from the dose–response curves for methacholine (73 (42–129) and 94 (66–134) $\mu g\ mL^{-1}$), 5-HT (1·5 (0·81–3·03) and 1·1 (0·51–2·24 $\mu g\ mL^{-1}$) and histamine (39 (21–75) and 72 (32–162) $\mu g\ mL^{-1}$) did not differ significantly ($P > 0.05$) between saline- and ovalbumin-challenged animals, respectively. However, when U-46619 was the spasmogen, ovalbumin-induced airway hyper-reactivity was observed as a leftwards shift of the concentration–response curve and the EC50 value for ovalbumin-challenged animals (8·1 (5·1–13) ng mL^{-1}) was significantly ($P < 0.05$) less than the value for control animals (39 (21–75) ng mL^{-1}).

Our findings suggest that airway hyper-reactivity is not 'non-specific', but instead depends on the chosen spasmogen. The absence of hyper-reactivity with certain spasmogens was not a result of poor delivery, because all spamogens caused a bronchoconstriction by the inhaled route. It was also not associated with the model because ozone has been shown to induce hyper-reactivity to inhaled methacholine and 5-HT. Because airway hyper-reactivity to both inhaled histamine and agonists at muscarinic receptors is regularly seen in man, the anaesthetized guinea-pig might not be the ideal model for assessing airway hyper-reactivity after antigen challenge and its modification by anti-asthma drugs.

The anaesthetized guinea-pig has been widely used as a model for asthma in man because of similarities between human asthma and allergen-induced changes in the guinea-pig. The underlying mechanism for both processes is the misdirected formation of IgE antibodies against innocuous antigens, leading, at renewed exposure, to an anaphylactic response. The response of guinea-pig airways to antigen challenge is in many ways similar to that in man, i.e. profound bronchoconstriction, increased influx of inflammatory cells (Kallos & Kallos 1984) and antigen-induced airway hyper-reactivity. In studies investigating this hyper-reactivity, the intravenous route has been chosen almost exclusively for spasmogen delivery. Because this route is less relevant to exposure in man, the current study uses the anaesthetized guinea-pig to investigate antigen-induced changes in airway reactivity to inhaled spasmogens. Other authors have described a method of aerosol delivery to anaesthetized guinea-pigs (Payne & De Nucci 1987) in which they compared intravenous and inhaled antigen challenges.

The anaesthetized animal might have some advantages over a conscious animal. The measurement of bronchospasm in an anaesthetized animal is often easier and more reproducible (less inter-animal variation) than plethysmographic monitoring of airway conductance (or resistance) in conscious animals. Furthermore, avoiding the upper airways (nasal passages, trachea, etc.) by introducing spasmogens directly into the lungs via the tracheal cannula reduces aerosol deposition outside the lung and thus increases its delivery to the central or peripheral airways, or both.

Typically, one or sometimes two spasmogens are used to assess airway reactivity; these are usually methacholine (or acetylcholine) and histamine. These spasmogens are the agents of choice for studying airway reactivity in man (where they are given by inhalation) and are used extensively for the demonstration of 'non-specific' airway hyper-reactivity after antigen challenge (Cockcroft et al 1977; Cartier et al 1982; Lai et al 1989; Rossi et al 1991). However, these agents have very similar mechanisms of action. Popa (1986) demonstrated that in man, histamine-induced bronchoconstriction was substantially but not exclusively mediated by the cholinergic pathway. Previous investigations of antigen-induced changes in airway reactivity to inhaled spasmogens have used conscious guinea-pig models and again have been confined to the use of these two spasmogens (Tarayre et al 1990; Griffiths-Johnson & Karol 1991; Asano et al 1994; Santing et al 1994; Yamada et al 1994). In this study, we assessed airway reactivity to four different inhaled spasmogens, methacholine, 5-hydroxytryptamine (5-HT), 9,11-dideoxy-9α,11α-methano-epoxy-$PGF_{2\alpha}$ (U-46619), a thromboxane A_2 mimetic, and histamine. Reactivity is equivalent to the sensitivity to the bronchoconstrictor activity of the spasmogen and is defined by the position of the concentration–response curve on the concentration axis. Thus, it is independent of any change in the magnitude of the response and in this study is measured at the 50% response (EC50).

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Komatsu & Takehana (1990) used the anaesthetized guinea-pig model to investigate airway reactivity immediately after antigen challenge; however, no studies have looked at airway reactivity associated with the late phase, which occurs approximately 18 h after the challenge. The aim of this work was to investigate whether an aerosolized antigen challenge resulted in increased airway reactivity (measured 18 to 24 h after challenge) in the anaesthetized guinea-pig.

Materials and Methods

Sensitization and challenge

Experiments were performed with male Dunkin-Hartley guinea-pigs, 400–500 g (Halls, Staffordshire). Each animal was sensitized with ovalbumin prepared according to the method described by Andersson (1980). Briefly, guinea-pigs received an intraperitoneal injection of ovalbumin (Sigma; 10 μg) and aluminium hydroxide (Rhone-Poulenc; 100 mg) suspended in normal saline (1 mL). After 14 to 21 days, animals were challenged with an ovalbumin macroshock (10 mg mL^{-1}) by inhalation. Mepyramine (10 mg kg^{-1} i.p.) was given 30 min before the challenge to protect against fatal anaphylaxis. Control animals were sensitized with ovalbumin but were challenged with a normal saline aerosol.

Determination of airway reactivity to inhaled spasmogens

Guinea-pigs were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}) by intraperitoneal injection 18 to 24 h after ovalbumin challenge and placed in the dorsal recumbent position on a heated small animal operating table. A cannula (Portex; o.d. 2·76 mm) was inserted into the trachea and the lungs were ventilated artificially using a Harvard constant-volume respiration pump (58 strokes min^{-1} of 1 mL laboratory air (100 g)$^{-1}$). The pulmonary inflation pressure (PIP) was measured with a pressure transducer (Pioden Controls Ltd) via a lateral port in the afferent limb of the ventilator circuit. To enable administration of drugs by inhalation, an ultrasonic nebulizer (PulmoSonic model 25, DeVilbiss) was used to deliver aerosols of spasmogens to the lungs. The nebulizer was connected in series with the afferent limb of the ventilator circuit and arranged such that inspired air passed through the nebulizer before entering the lungs via the tracheal cannula (Payne & De Nucci 1987). Concentration–response curves were obtained by successively increasing the concentration of the inhaled spasmogen. The duration of aerosol delivery was kept constant at 40 s. Systemic arterial blood pressure (BP) measurements were made via a polythene catheter (Portex; o.d. 0·96 mm) placed in a carotid artery (usually the right) and connected to a Bell and Howell physiological-pressure transducer. Blood pressure and PIP were recorded using a Lectromed MT8P (Welwyn, UK) recorder. The lung-pressure circuit was calibrated by total occlusion of the tracheal cannula directly before each experiment and PIP measurements were expressed as a percentage of the maximum possible bronchoconstriction.

During a period of equilibration, typically 10 min, after surgical intervention the lungs were hyper-inflated with 3 tidal volumes of room air (by occluding outflow) and left to reach a stable resting PIP value. Non-cumulative concentration–response curves were obtained for methacholine, 5-HT, U46619 and histamine (Sigma, UK). Doses were given at approximately 6 min intervals after BP had re-stabilized and PIP had returned to pre-constriction baseline levels. At the end of each experiment an aerosol of ovalbumin (0·1 mg mL^{-1}) was given to verify sensitization. Animals not responding with bronchoconstriction (<10%) were excluded from the study and are not included in the mean data shown in Fig. 1 and Tables 1 and 2.

To assess the possible site of action of each spasmogen, the extent of recovery of the bronchoconstriction by hyper-inflation of the lungs was measured according to the method of Tokuyama et al (1991). Five minutes after inhalation of the largest concentration of spasmogen, the lungs were hyper-inflated by means of a brief occlusion of the outflow of the ventilator. The recovery was measured at 6 min by subtracting the pre-inhalation baseline PIP from the 6 min PIP value and expressing this as a percentage of the maximum possible recovery (the difference between the PIP at 5 min and baseline).

Generation of spasmogen aerosols

An ultrasonic nebulizer (PulmoSonic model 5000I, DeVilbiss) was used to deliver aerosols to the lungs via the tracheal cannula. Droplet size was measured using a laser-light-scattering device (Malvern Instruments 2600 HSD analyser) and the mass median aerodynamic diameter was found to range between 4·26 and 4·78 μm. These values were obtained from measurements of drug solutions in normal saline (the vehicle) and from the vehicle alone. The average percentage of droplets < 5 μm in diameter was $77{\cdot}5 \pm 6{\cdot}1$.

Blood-pressure measurement

Blood pressure was routinely monitored in control and ovalbumin-challenged guinea-pigs. The effect of the spasmogen on BP was used to confirm (indirectly) its absorption from the airways. To compare control and ovalbumin-challenged animals, effective concentrations (EC) for an arbitrary change in diastolic blood pressure of 10 mm Hg were calculated (EC10). These values were calculated for an agent causing a fall in diastolic pressure (histamine) and one causing an increase in blood pressure (U-46619). Significant differences between EC10 values were then determined by use of Student's *t*-test. It is theoretically possible that the change in BP seen after the inhalation of spasmogens might be secondary to the bronchoconstriction itself. This was not thought to be so in these experiments as spasmogens gave rise to either increases or decreases in BP. Furthermore, in some instances blood pressure changes were seen in the absence of bronchoconstriction.

Data analysis

Baseline PIP values were not significantly different ($P > 0{\cdot}05$) between groups and so the peak PIP values (expressed as a percentage of the maximum possible PIP) were used for construction of concentration–response curves. Log concentration–response curves were constructed from PIP values and the data points shown are means ± s.e.m. Geometric mean EC50 values (concentration required for bronchoconstriction equivalent to 50% maximum PIP) were determined together with their 95% confidence limits. These values were analysed using an unpaired Student's *t*-test to determine whether differences between control and treated groups were significant. Airway hyper-reactivity was therefore defined in this study as a

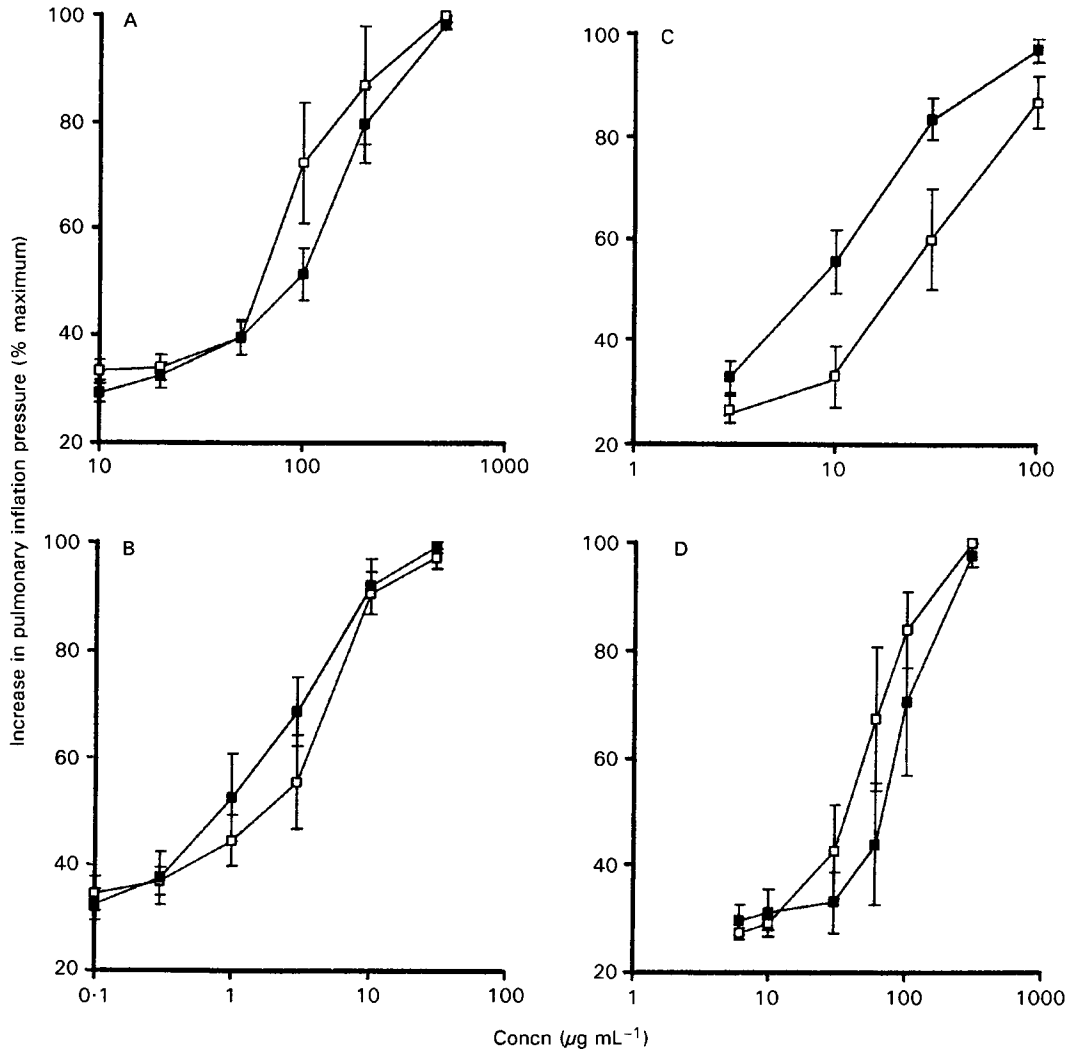

FIG. 1. Mean concentration–response curves for the increases in pulmonary inflation pressure (PIP) of anaesthetized guinea-pigs after inhalation of spasmogen. Peak PIP values are expressed as a percentage of the maximum possible PIP in response to (A) methacholine; (B) 5-HT; (C) U-46619 and (D) histamine. Responses of sensitized guinea-pigs were obtained 24 h after an aerosol challenge with either saline (□) or ovalbumin (■). Each point is the mean ± s.e.m. of n values as indicated in Table 1.

significant leftwards shift of the concentration–response curve for the inhaled spasmogen.

Results

Inhaled normal saline rarely caused an increase in baseline PIP and on occasions when this did occur, the change was less than a 5% increase. In contrast, administration of spasmogens by the inhaled route caused concentration–dependent bronchoconstriction. Table 1 compares data obtained from anaesthetized guinea-pigs 18 to 24 h after saline or ovalbumin challenge. When methacholine, 5-HT or histamine was the spasmogen, ovalbumin-induced airway hyper-reactivity was not observed because the concentration-response curves were either superimposed or the curves from challenged groups lay to the right of controls (non-significant) (Figs 1a, b and d, respectively). Airway hyper-reactivity was, however, observed when U-46619 was the spasmogen, the curve after ovalbumin challenge being shifted significantly to the left (Fig. 1c).

The effects of inhaled spasmogens on diastolic BP were similar in control and ovalbumin-challenged guinea-pigs. The EC10 values for the increases in diastolic BP after inhaled U-46619, 17 ± 3·7 and 19 ± 4·8 ng mL^{-1} in control and ovalbumin-challenged animals, respectively, were not significantly different ($P = 0{\cdot}71$). Similarly, the values for the falls in diastolic BP after histamine challenge, −135 ± 20 and −94 ± 19 μg mL^{-1}, in control and ovalbumin-challenged animals, respectively, were also not significantly different ($P = 0{\cdot}19$).

Table 1. Airway reactivity to inhaled spasmogens assessed 18–24 h after challenge of sensitized guinea-pigs with saline or ovalbumin.

	Mean EC50 (95% confidence limits; μg mL^{-1})				
Spasmogen	Saline	n	Ovalbumin	n	*P*-value
Methacholine	73 (42–129)	4	94 (66–134)	8	0·44
5-HT	1·5 (0·81–3·03)	6	1·1 (0·51–2·24)	7	0·49
U-46619	17 (11–28)†	4	8·1 (5·1–13)†	6	0·038*
Histamine	39 (21–75)	5	72 (32–162)	5	0·26

*Significant difference between results from saline and ovalbumin.
†EC50 values in ng mL^{-1} rather than μg mL^{-1}.

Table 2. Recovery, induced by hyper-inflation of the lungs, of anaesthetized guinea-pigs from bronchoconstriction to inhaled spasmogen.

	Mean percentage recovery (±s.e.m.)			
Spasmogen	Saline	n	Ovalbumin challenge	n
Saline	92·9±5·2	4	95·5±9·1	7
Methacholine	42·1±9·2	4	44·5±6·3	7
5-HT	36·6±6·9	6	27·0±2·7	6
U-46619	13·4±3·8	4	11·1±1·7	6
Histamine	67·1±8·2	5	66·3±3·5	5

Recovery was measured 6 min after inhalation of the highest concentration of spasmogen.

The recovery from the bronchoconstriction induced by each spasmogen was improved by hyper-inflation of the lungs. The percentage recovery values after hyper-inflation of saline- and ovalbumin-challenged animals are shown in Table 2. There was no significant difference ($P > 0.05$) in recovery between saline and ovalbumin challenge with any of the spasmogens. The recovery from the U46619-induced bronchoconstriction was significantly slower than for histamine or methacholine in the ovalbumin-challenged animals.

Discussion

We have demonstrated that it is not always possible to show ovalbumin-induced airway hyper-reactivity to inhaled spasmogens in the anaesthetized guinea-pig model. An increase in airway reactivity was seen with U-46619 but not with methacholine, 5-HT or histamine. The occurrence of hyper-reactivity with U-46619 confirms that the animals could show airway hyper-reactivity 18 to 24 h after ovalbumin challenge.

Aerosol droplet size is one of the factors which influences where deposition is likely to occur in the airways (Lippmann 1977). In the current study the aerosol droplet mass median aerodynamic diameter was approximately 4·5 μm. Although ideally a smaller particle size would have been desirable, no problems were encountered in obtaining bronchoconstriction with these aerosols, and other studies in the guinea-pig have successfully used inhaled particles of a similar size (Underwood et al 1991; Yamada et al 1994). Furthermore, ozone-induced airway hyper-reactivity has been observed after challenge with methacholine or 5-HT inhaled under identical experimental conditions in our laboratories (Johnson et al 1997).

When spasmogens are inhaled, the bronchoconstriction which follows might arise from the stimulation of receptor sites which are more central in the airway than those which were stimulated by an intravenous dose of the same spasmogen. After intravenous administration, spasmogens such as histamine, acetylcholine and 5-HT first reach the airway via the pulmonary circulation which supplies the peripheral airways (respiratory bronchioles and alveolar ducts) where stimulation of appropriate sites results in an instantaneous constrictor response (Colebatch et al 1966). These route differences might contribute to the lack of ovalbumin-induced airway hyper-reactivity in this study as the site of reactivity changes might be more peripheral and thus not susceptible to measurement after inhalation of methacholine, 5-HT and histamine. U-46619 might penetrate further into the airways than the other spasmogens and reveal an increase in airway reactivity at a more peripheral site. Attempts were made to find the approximate site of action of spasmogens using the method described by Tokuyama et al (1991) in which hyper-inflation of the lungs is used to assist recovery after spasmogen-induced bronchoconstriction. Hyper-inflation preferentially reverses airway narrowing caused by contraction of airway smooth muscle (Tokuyama et al 1991) whereas changes in the central airways are less affected. Rapid and complete recovery would therefore suggest a relatively peripheral site of bronchoconstriction whereas a poorer recovery would indicate constriction in the more central airways. However, interpretation is not straightforward for spasmogens such as U-46619, which also stimulate an increase in secretions (Tokuyama et al 1991). This action might mask an otherwise good recovery, indicating the site of action to be central whereas the actual site of action might be peripheral. Indeed, the bronchoconstriction resulting from U-46619 was not as rapid as might be expected of a peripherally acting spasmogen. Thus, hyper-inflation failed to confirm that the difference in reactivity changes after ovalbumin challenge was a consequence of differences in their sites of action. Nevertheless, it was clear that hyper-reactivity was observed with the spasmogen (U-46619) which displayed the slowest reversal by hyper-inflation.

It is clear that our results do not support the idea that airway hyper-reactivity is a 'non-specific' phenomenon. During the course of this study there has been a steady increase in the number of publications supporting a more selective kind of hyper-reactivity in man. Haugaard et al (1992) subjected atopic asthmatics to challenges with histamine, antigen, distilled water and exercise. They found that for these challenges there were different mechanisms or pathways leading to bronchoconstriction. This view was upheld by Djukanovic (1993) who stated that close correlations between responses to histamine and methacholine alone do not constitute 'non-specific' airway responsiveness. If the phenomenon were truly non-specific it could be applied to stimuli other than spasmogens, but that is not so. Also, the allergen caused a greater and more prolonged increase in responsiveness to bradykinin than to methacholine, thus supporting the idea that hyper-responsiveness is a selective pathophysiological abnormality (Djukanovic 1993). Differences in airway reactivity to inhaled methacholine and bradykinin were also demonstrated by Berman et al (1995) in asthmatic patients. They found a greater extent of airway hyper-reactivity to bradykinin than methacholine when measurements were made 24 h after antigen challenge. In addition, the development of airway hyper-reactivity for the two agonists followed different time-courses, maximum reactivity to methacholine was generally seen after approximately 6 to 8 h (airway reactivity to methacholine was not significantly increased after 24 h) whereas airway hyper-reactivity to bradykinin was maximum after approximately 48 h. Several studies have shown the reactivity of leukotrienes to be different from those of methacholine or histamine. Aspirin-sensitive asthmatics show selective hyper-responsiveness to LTE_4 but not to LTC_4 or histamine (Christie et al 1993) and Adelroth et al (1986) found differences in reactivity between methacholine and both LTC_4 and LTD_4, i.e. asthmatics who were most responsive to methacholine were least responsive to both leukotrienes.

In a study which selected known late responders, Ward et al (1994) were unable to show antigen-induced airway hyper-reactivity after 24 h using methacholine. This was attributed to high baseline responsiveness, although this is hard to reconcile for 20 individuals with mild stable asthma, normally maintained on, at most, inhaled β_2-agonists. The extent of antigen-induced airway hyper-reactivity to inhaled methacholine seen in the work of Cockcroft et al (1977) was not particularly great, with only six out of a possible thirteen subjects showing airway hyper-reactivity after 8 h. Furthermore, they showed a difference in the time-course of airway hyper-reactivity revealed by aerosols of methacholine (8 to 32 h) and histamine (8 to 56 h). Aalbers (1991) demonstrated a difference in the time-course of antigen-induced airway hyper-reactivity using aerosols of methacholine and the indirectly acting adenosine 5′-monophosphate. Significant airway hyper-reactivity was seen after 3 h with both agonists, but after 24 h with methacholine only, suggesting that several mechanisms might be involved.

Our findings suggest that changes in ovalbumin-induced airway reactivity were not 'non-specific' but instead appeared to depend on the chosen spasmogen. It is not clear why ovalbumin-induced airway hyper-reactivity to inhaled 5-HT was not seen (despite good spasmogen responses having been attained from aerosol delivery) as airway hyper-reactivity was evident when the same spasmogen was given intravenously (Johnson & Broadley 1995). The absence of ovalbumin-induced airway hyper-reactivity is not associated with the model itself because ozone-induced airway hyper-reactivity has been shown in response to inhaled methacholine and 5-HT (Johnson et al 1997). The reasons for this difference are not clear.

The results from this work with inhaled spasmogens show that ovalbumin-induced airway hyper-reactivity is not easy to demonstrate in the anaesthetized guinea-pig. This differs significantly from the situation in man for which airway hyper-reactivity both to inhaled histamine and to agonists at muscarinic acetylcholine receptors is regularly seen. It therefore seems that the anaesthetized guinea-pig model might not be ideal for assessment of airway hyper-reactivity and its modification by anti-asthma drugs.

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