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Airway hyper- or hyporeactivity to inhaled spasmogens 24 h after ovalbumin challenge of sensitized guinea-pigs

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1 The aim of this study was to determine whether an inhalation of ovalbumin (OA, 10 or 20 mg ml^{-1}) by conscious OA-sensitized guinea-pigs leads to airway hyperreactivity to spasmogens 24 h later. In contrast to most previous studies, the spasmogens (5-HT, methacholine (MCh), U-46619 and adenosine) were administered by inhalation and airway function was measured in conscious guinea-pigs.

2 Guinea-pigs were sensitized by i.p. injection of 10 μg OA and 100 mg aluminium hydroxide in 1 ml normal saline; 14–21 days later they were exposed to an inhalation of 5-HT, MCh, U-46619 or adenosine. Specific airway conductance (sG_{aw}) was measured in conscious animals by whole body plethysmography. The spasmogens caused bronchoconstriction, measured as a reduction in sG_{aw} from the pre-inhalation basal values. Dose-related bronchoconstrictions were observed with 5-HT, MCh and U-46619.

3 The effect of an ovalbumin macroshock challenge upon the responses to each spasmogen were examined by giving an inhalation of aerosolized OA at 24 h (or 7 days in the case of adenosine) after an initial spasmogen challenge. Eighteen to twenty-four hours after the OA macroshock, the same guinea-pigs were exposed to a repeated inhalation of 5-HT, MCh, U-46619 or adenosine.

4 U-46619 was the only spasmogen to demonstrate hyperresponsiveness, the peak change in sG_{aw} being increased from -12.3 ± 9.9 to $-38.8 \pm 5.0\%$ by 10 mg ml^{-1} OA challenge. In contrast, the ovalbumin challenge (20 mg ml^{-1}) inhibited the bronchoconstrictions to 5-HT (50 μg ml^{-1}) and MCh (100 μg ml^{-1}). Adenosine demonstrated bronchoconstriction in sensitized guinea-pigs but no significant change in the response was observed after an OA challenge.

5 All results were compared with a control group of sensitized guinea-pigs receiving a NaCl challenge. The bronchoconstrictor responses to 5-HT, MCh, U-46619 or adenosine did not differ significantly before and after the saline challenge, indicating reproducibility of the responses.

6 In further experiments, guinea-pigs were exposed to inhalation of 5-HT (50 μg ml^{-1}) or MCh (300 μg ml^{-1}) 24 h before atropine (10 μg, 100 μg or 1 mg ml^{-1}) and again at 0.5 to 1.5 h afterwards. Atropine antagonized the 5-HT- and MCh-induced bronchoconstrictions over the same antagonist dose-range. This suggests that the bronchoconstriction induced in the conscious guinea-pig by 5-HT is mediated primarily via muscarinic receptors, possibly by a vagal reflex. The inhibition of the responses to 5-HT and MCh by OA challenge would therefore appear to be related to interference with a common cholinergic pathway for these spasmogens.

7 In summary, airway hyperresponsiveness was evident at 24 h after OA challenge as measured by an enhanced bronchoconstrictor response to inhaled U-46619. When 5-HT or MCh were used as the spasmogens, an opposing decrease in responsiveness was observed. This was presumed to be due to an inhibition of cholinergic pathways by the OA challenge. Adenosine caused a bronchoconstriction in the sensitized animals but this was not enhanced by the OA challenge.

Keywords: Conscious sensitized guinea-pigs; ovalbumin; spasmogens; hyperresponsiveness; hyporesponsiveness; atropine blockade; vagal reflex

Introduction

Airway hyperresponsiveness to bronchoconstriction by spasmogens is a hallmark of the asthmatic patient (Boushey *et al.*, 1980). It is well known that susceptible individuals become sensitized to various proteins and go on to develop atopic asthma and associated airway hyperreactivity on subsequent exposures. The development of animal models of airway hyperreactivity is a common approach to investigate the possible mechanisms involved in the pathogenesis of this phenomenon and additionally, allow the development of novel therapeutic agents which may be of benefit in asthma.

Raeburn *et al.* (1992) reviewed a variety of techniques to measure parameters of lung function in small laboratory animals. These include models in which bronchospasm is assessed in anaesthetized, mechanically ventilated animals (Payne & DeNucci, 1987). These techniques are widely used but because they require surgical intervention they are not suited to long-term or repeat studies. Bronchoconstrictors, including 5-hydroxytryptamine (5-HT) and acetylcholine, are commonly used to demonstrate airway hyperreactivity by i.v. or inhalation administration in anaesthetized guinea-pigs following bronchial anaphylaxis (Daffonchio *et al.*, 1987; 1989; Coyle *et al.*, 1988; Masaki *et al.*, 1994). Sanjar *et al.*. (1990) demonstrated airway hyperreactivity to i.v. histamine up to 48 h and to i.v. prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) up to 12 h after aerosolized antigen challenge in sensitized guinea-pigs.

Non-surgical plethysmographic techniques allow the long-term or repeated measurement of lung function in conscious animals under minimal restraint (Amdur & Mead, 1958; Griffiths-Johnson *et al.*, 1988; Ball *et al.*, 1991; Thorne & Broadley, 1994). The technique developed by Griffiths-Johnson *et al.* (1988) is the basis of the method used in this study. Methacholine (MCh) is the bronchoconstrictor commonly used to demonstrate bronchial hyperreactivity in human studies of asthma (Durham & Kay, 1985) and chronic obstructive pulmonary disease (COPD) (Taylor *et al.*, 1985; Roberts *et al.*, 1983). However, studies demonstrating bronchial hyper-

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reactivity in conscious guinea-pigs vary considerably in their methodology and are, like human studies, limited in their use of spasmogens. Tarayre *et al.* (1990) demonstrated bronchoconstriction and convulsions to inhaled histamine in conscious, sensitized guinea-pigs at 1, 3, 6, 24 and 48 h following a challenge to aerosolized ovalbumin or saline. Bronchial hyperreactivity to histamine was only observed at 3 and 6 h after the ovalbumin challenge. Griffiths-Johnson *et al.* (1991) demonstrated airway hyperreactivity to histamine 1 h after an aerosolized ovalbumin challenge in conscious, sensitized guinea-pigs. In contrast, Featherstone *et al.* (1988) investigated bronchial reactivity to histamine and methacholine in guinea-pigs sensitized, but not challenged, to ovalbumin. Guinea-pigs were sensitized with two aerosolized challenges of ovalbumin for 3 min, one week apart. Responsiveness to histamine and methacholine was investigated one week after the second ovalbumin challenge. Therefore, full exploitation of conscious guinea-pigs to assess airway reactivity by whole body plethysmography has not yet been made, only histamine having been examined. Histamine may have limitations where mepyramine is used to protect from fatal anaphylaxis during the antigen challenge.

Thus, the present study was undertaken to evaluate a small range of other spasmogens to determine whether hyperreactivity occurs after challenge of sensitized guinea-pigs with aerosolized antigen. The inhaled spasmogens were 5-HT, methacholine (MCh), the thromboxane-mimetic, U-46619 and adenosine. They were selected because of their different sites of action to provide information on the possible underlying mechanisms of airway hyperreactivity in subjects with atopic asthma. For consistency with earlier studies, a single 'macroshock' challenge with ovalbumin was used in the present study. This would establish whether hyperreactivity could be induced and would provide a basis for future studies using chronic exposures which would be of more relevance to the situation in human asthma.

Methods

Male Dunkin-Hartley guinea-pigs were actively sensitized on day 1 by i.p. injection of 10 μg ovalbumin and 100 mg aluminium hydroxide in 1 ml normal saline (Andersson, 1981); 14–21 days later the animals were used to measure the effects of inhaled spasmogens by whole body plethysmography.

Exposure to spasmogens

An initial (basal) value of specific airways conductance (sG_{aw}) (see below) was recorded. The guinea-pigs were then exposed to nebulized aerosols of 5-HT, MCh, U-46619 or adenosine in a sealed, wooden box (measuring 350 × 200 × 150 mm), for 1 min. At either end of the box there was a hole (approx. 8 mm diameter), one for the administration of the aerosols, the other acting as an exhaust. The atmosphere in the exposure box was allowed to become saturated with the aerosol for 2 min before exposing the animal. The aerosol was generated by a Wright nebuliser using medical air delivered at 20 psi. Delivery of the aerosol was calculated to be 2 ml min^{-1}. Changes in sG_{aw} were measured at 2, 5, 10 and 20 min after the control exposure.

Dose-response relationships

Three doses of the spasmogens (5-HT, MCh or U-46619) were examined on consecutive days in the same animal.

Effect of ovalbumin challenge

On the day after a challenge with 5-HT, MCh or U-46619, the same animals were exposed to a macroshock (10 mg or 20 mg ml^{-1}) of ovalbumin in 0.9% NaCl, by inhalation in a sealed box for 2 min. In the case of adenosine, the animals were challenged with 10 mg ml^{-1} OA, 7 days after the initial adenosine exposure. The doses of spasmogens were selected from dose-response data shown in this paper (Figure 1) for 5-HT, MCh and U-46619 and previously for adenosine (Thorne & Broadley, 1994) to yield submaximal yet measurable responses. Animals were protected against fatal anaphylaxis with mepyramine (30 mg kg^{-1}, i.p.) administered 30 min before OA exposure. To determine whether any hyperreactivity was present, the same group of guinea-pigs was exposed to an aerosol of 5-HT, MCh, U-46619 or adenosine, as before, at 18–24 h after the ovalbumin macroshock. These results were compared with a control group receiving an aerosol of NaCl for 2 min instead of ovalbumin.

Effect of atropine

To investigate the susceptibility of inhaled 5-HT and MCh to blockade with atropine, sensitized guinea-pigs received a control challenge inhalation of either 5-HT (500 μg ml^{-1}) or MCh (300 μg ml^{-1}). The following day atropine (1 mg, 100 μg or 10 μg kg^{-1}) was administered (i.p.) between 0.5 and 1.5 h before repeating the inhalation challenge in the same group of animals. Whereas threshold responses were required for the hyperreactivity studies, more substantial responses using larger doses were used for these antagonism studies.

Non-invasive measurement of specific airways conductance

Specific airways conductance (sG_{aw}) was determined using constant volume, whole body plethysmography in unanaesthetized spontaneously breathing guinea-pigs (Agrawal, 1981; Griffiths-Johnson *et al.*, 1988; Thorne & Broadley, 1994). Each animal was fitted with a face mask and placed in a restrainer which was then slid into the plethysmograph chamber. Respiratory airflow was measured with a pneumotachograph (Mercury FIL) via the face mask. When the thermal drift of the plethysmograph had stabilized, the chamber was sealed. During breathing, the alveolar pressure difference (i.e. the pressure difference between mouth and alveoli needed for airflow to occur) acts on the small volume change that is measured as a change in pressure in the plethysmograph. Box pressure and respiratory airflow were measured with pressure transducers (UP1 and UP2 respectively) and fed into a storage oscilloscope to generate a pressure-flow loop. The angle, θ, between the vertical and linear phase (near zero flow at end expiration) was used to calculate sG_{aw}. This angle was measured by superimposing an electronic angle cursor, generated by a purpose built 'resolver' (Engineering workshop SmithKline Beecham, The Frythe, Welwyn Garden City, Herts). Four measurements were taken at each time point, and the guinea-pigs removed after each reading. Prior to each experiment, the guinea-pigs were handled and familiarized with the restrainer and the plethysmograph chamber; this reduced any stress placed on the animals and reduced the variability of the results.

Drugs and solutions

Atropine sulphate, adenosine, 5-hydroxytryptamine (3-(2-aminoethyl)-5-hydroxyindole; serotonin), methacholine bromide (acetyl-β-methylcholine bromide), ovalbumin and U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) were all purchased from Sigma, Poole, Dorset.

Aluminium hydroxide ($Al(OH)_3$) and mepyramine maleate were gifts from Rhône-Poulenc-Rorer, Dagenham, Essex.

Data analysis

The time-course of the bronchoconstrictor response to the spasmogen was plotted as the % change in specific airway conductance (sG_{aw}) from the pre-exposure basal value. The mean (±s.e.mean) values at each time point were calculated.

Statistical comparisons were made between the peak values (2 or 5 min) of sG_{aw} before and after an ovalbumin challenge by means of Student's paired t test. To quantify the degree of blockade of the bronchoconstrictor responses to 5-HT and MCh, peak bronchoconstriction at 2 min was measured as the change in specific airway conductance (sG_{aw}), from the baseline value. The value after atropine injection was expressed as a percentage of the pre-atropine value. This value was then subtracted from 100 and the mean (±s.e.mean) used as a measure of the degree of inhibition by atropine. Statistical comparisons between sG_{aw} values (% change) before and after atropine were made at 2 min by Student's paired t test and at both 2 and 5 min by Analysis of Variance (ANOVA). $P<0.05$ was considered significant.

Results

Dose-related effects of spasmogens

Inhalation of nebulized solutions of 5-HT, MCh and U-46619 caused dose-related bronchoconstrictions, measured as a reduction in sG_{aw} of conscious, sensitized guinea-pigs ($n=4$). The peak occurred at approximately 2 min and the baseline was restored by 20 min (Figure 1). Adenosine (1 mg ml^{-1}) also caused bronchoconstriction which our previous studies have shown to be a submaximal dose (Thorne & Broadley, 1994).

Effects of ovalbumin challenge on spasmogen responses

Experiments were next performed to examine the bronchoconstriction induced by 50 μg ml^{-1} 5-HT or 100 μg ml^{-1} MCh before and 18–24 h after a macroshock with inhaled ovalbumin (20 mg ml^{-1}). The reductions in sG_{aw} by 5-HT and MCh before challenge peaked at 2 min (−65.6±10.1 and −57.5±12.1% of basal sG_{aw} respectively, $n=4$). At 18–24 h after the macroshock, the bronchoconstriction was significantly reduced ($P<0.05$), the sG_{aw} values peaked at 5 min and were −13.5±11.0 and −19.3±5.2%, respectively, of basal values (Figure 2). When the concentration of 5-HT was reduced to 10 μg, a small threshold bronchoconstriction of −10.9±10.2% peaking at 5 min was obtained ($n=4$). Again, there was no increase in the magnitude of this effect after the OA challenge (Figure 2b).

In contrast, the bronchoconstriction to U-46619 (0.03 μg ml^{-1}), was significantly potentiated ($P<0.05$) at 18–24 h after macroshock with either 10 mg or 20 mg ml^{-1} OA. The reductions of sG_{aw} prior to OA challenge peaked at 5 min with values of −12.3±9.9 and −22.1±5.3% ($n=6$) of baseline, respectively. The values after challenge with 10 and 20 mg ml^{-1} OA were significantly enhanced ($P<0.05$) to −38.8±5.0 and −38.1±3.9%, respectively (Figure 3).

The adenosine (1 mg ml^{-1})-induced bronchoconstriction peaked at 5 min, the fall in sG_{aw} value being −30.7±6.7% of baseline. After 7 days, a macroshock of OA was given and 18–24 h later, the response to adenosine (−27.6±6.2%, $n=6$) was virtually superimposed on the pre-challenge response (Figure 4).

Control experiments

The effects of OA macroshock were compared with a control group of sensitized animals receiving an NaCl challenge instead of ovalbumin. The bronchoconstrictor responses to 5-HT (50 μg ml^{-1}), MCh (100 μg ml^{-1}) and U-46619 (0.03 μg ml^{-1}) were obtained 24 h before and again 18–24 h after the NaCl challenge. The responses were not significantly different (Figure 5). In the case of adenosine, the responses were obtained 8 days apart, the second inhalation being given 18–24 h post-NaCl. Again the responses were not significantly different (Figure 5).

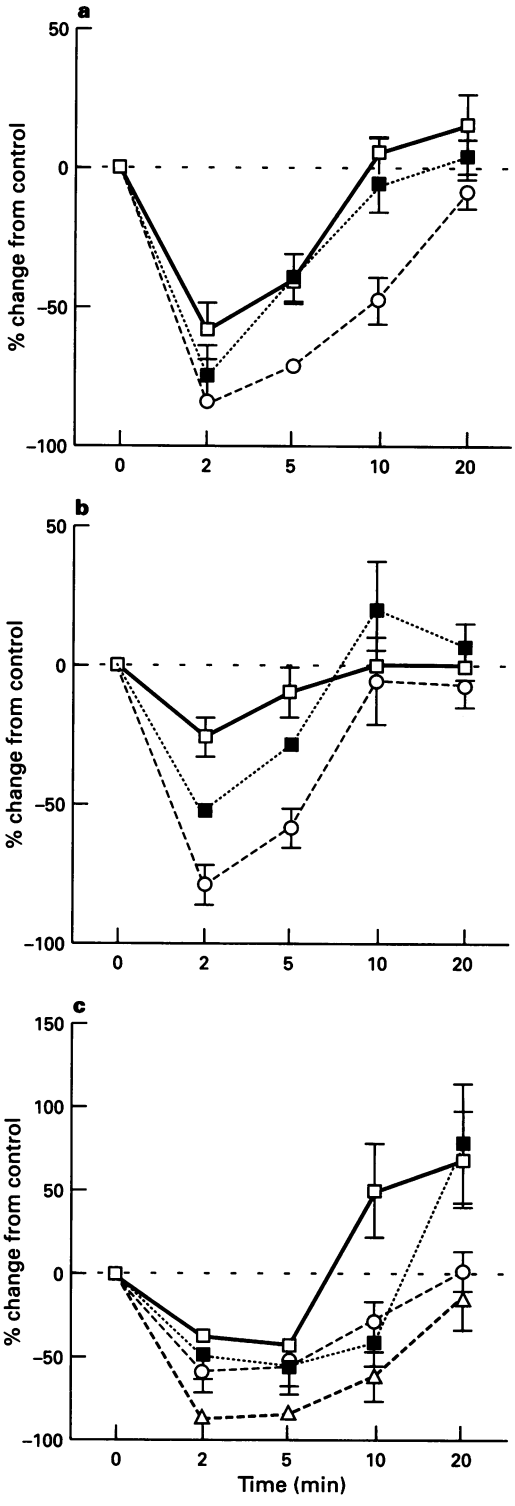

Figure 1 Time courses for bronchoconstriction by increasing concentrations of inhaled 5-HT (a), methacholine (b) and U-46619 (c) in conscious, sensitized guinea-pigs. In (a) and (b): (□) 30 μg ml^{-1}; (■) 100 μg ml^{-1}; (○) 300 μg ml^{-1}; In (c) (□) 0.03 μg ml^{-1}; (■) 0.1 μg ml^{-1}; (○) 0.3 μg ml^{-1}; (△) 1 μg ml^{-1}. Bronchoconstriction is measured as the mean (±s.e.mean) fall in sG_{aw} expressed as a % of the basal pre-inhalation value ($n=4$).

Effect of atropine on 5-HT- and MCh-induced bronchoconstrictions

The changes in sG_{aw} for 5-HT (50 μg ml^{-1}) and MCh (300 μg ml^{-1}) measured at 2 min were reduced by 88.0±12.1 and 69.9±31.4% after atropine (1 mg kg^{-1}) (Figure 6, Table 1). Similarly, virtually identical blockade of 5-HT and MCh

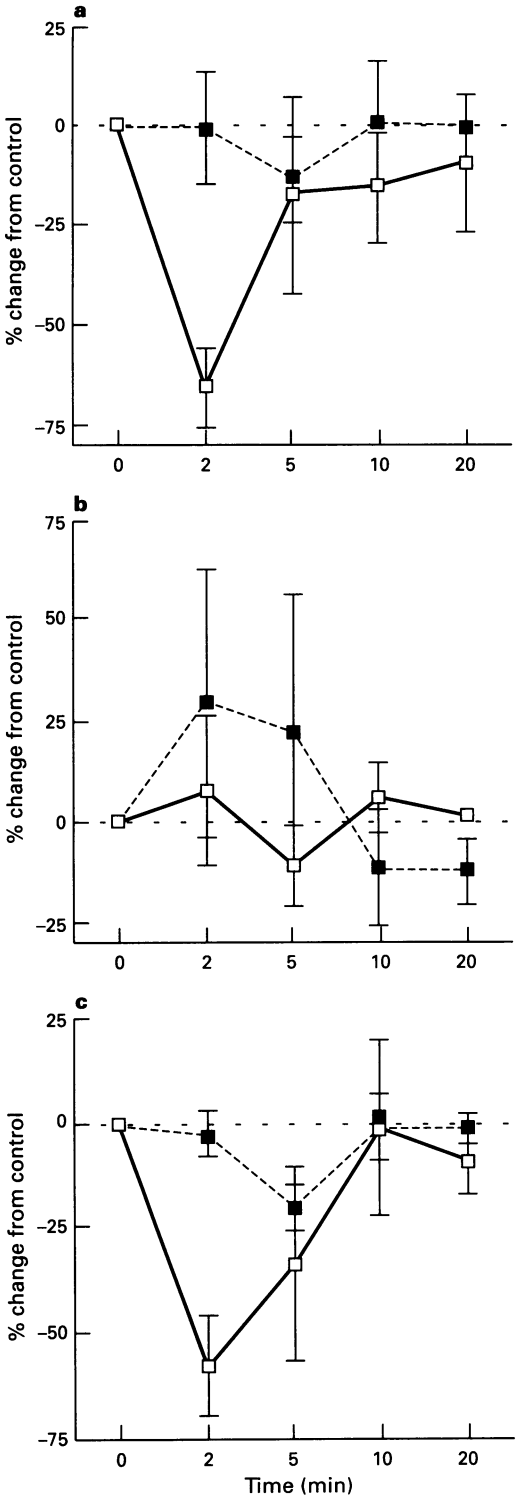

Figure 2 Effects of inhalation exposure to OA (20 mg ml^{-1}) of conscious OA-sensitized guinea-pigs upon the bronchoconstrictor responses to 50 μg ml^{-1} 5-HT (a), 10 μg ml^{-1} 5-HT (b) and 100 μg ml^{-1} methacholine (c). Animals were exposed to inhalation of 5-HT or methacholine 24 h before OA challenge (□) and again at 18–24 h after the challenge (■). Changes in sG_{aw} value are expressed as the mean ± s.e.mean ($n = 4$) percentage change from the pre-inhalation basal value.

was obtained with 100 μg kg^{-1} atropine where inhibitions at the 2 min time point were 57.4 ± 27.2 and 56.6 ± 24.7%, respectively (Figure 6 and Table 1). A further reduction of the dose of atropine to 10 μg kg^{-1} still inhibited the 5-HT-induced change in sG_{aw}, measured at 2 min, by 53.6 ± 7.5% (Table 1). Measured at the 2 min time point, only the blockade of 5-HT

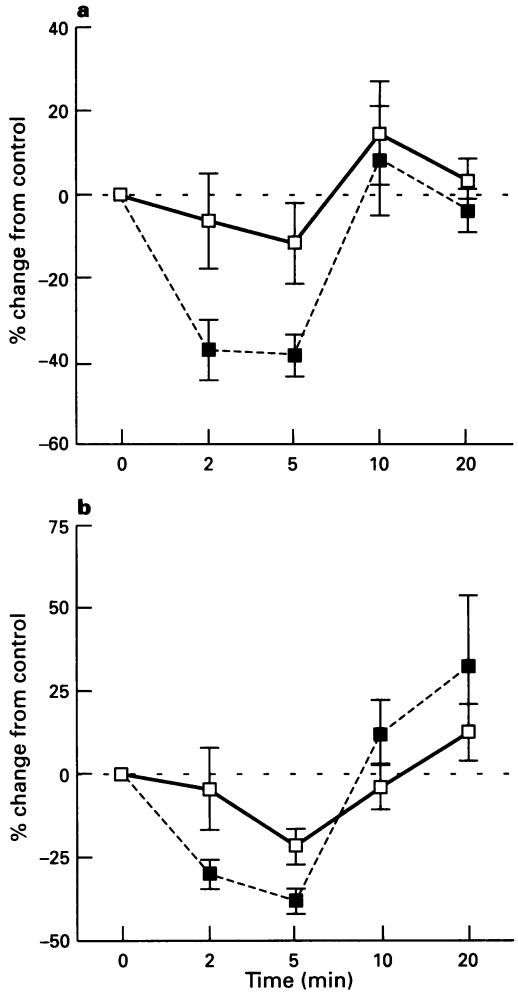

Figure 3 Effects of inhalation exposure to OA (10 mg ml^{-1}, a; or 20 mg ml^{-1}, b) of conscious OA-sensitized guinea-pigs upon bronchoconstrictor responses to inhaled U-46619 (0.03 μg ml^{-1}). Animals were exposed to inhalations of U-46619 24 h before OA challenge (□) and again at 18–24 h after the challenge (■). Changes in sG_{aw} values are expressed as the mean ± s.e.mean ($n = 6$) percentage change from the pre-inhalation basal value.

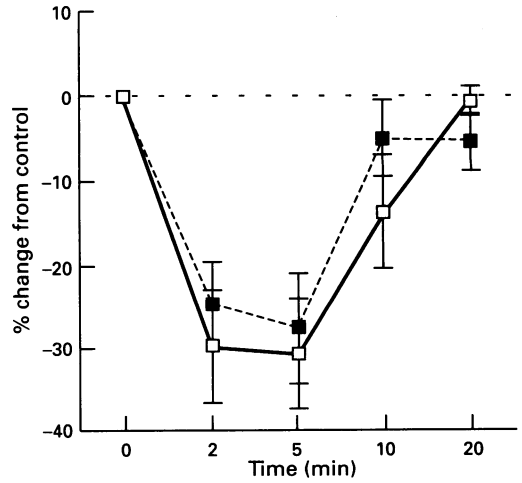

Figure 4 Effect of inhalation exposure to OA (10 mg ml^{-1}) of conscious OA-sensitized guinea-pigs upon bronchoconstrictor responses to inhaled adenosine (1 mg ml^{-1}). Animals were exposed to inhalations of adenosine 7 days before OA challenge (□) and again at 18–24 h after the challenge (■). Changes in sG_{aw} values are expressed as the mean ± s.e.mean ($n = 6$) percentage change from the pre-inhalation basal value.

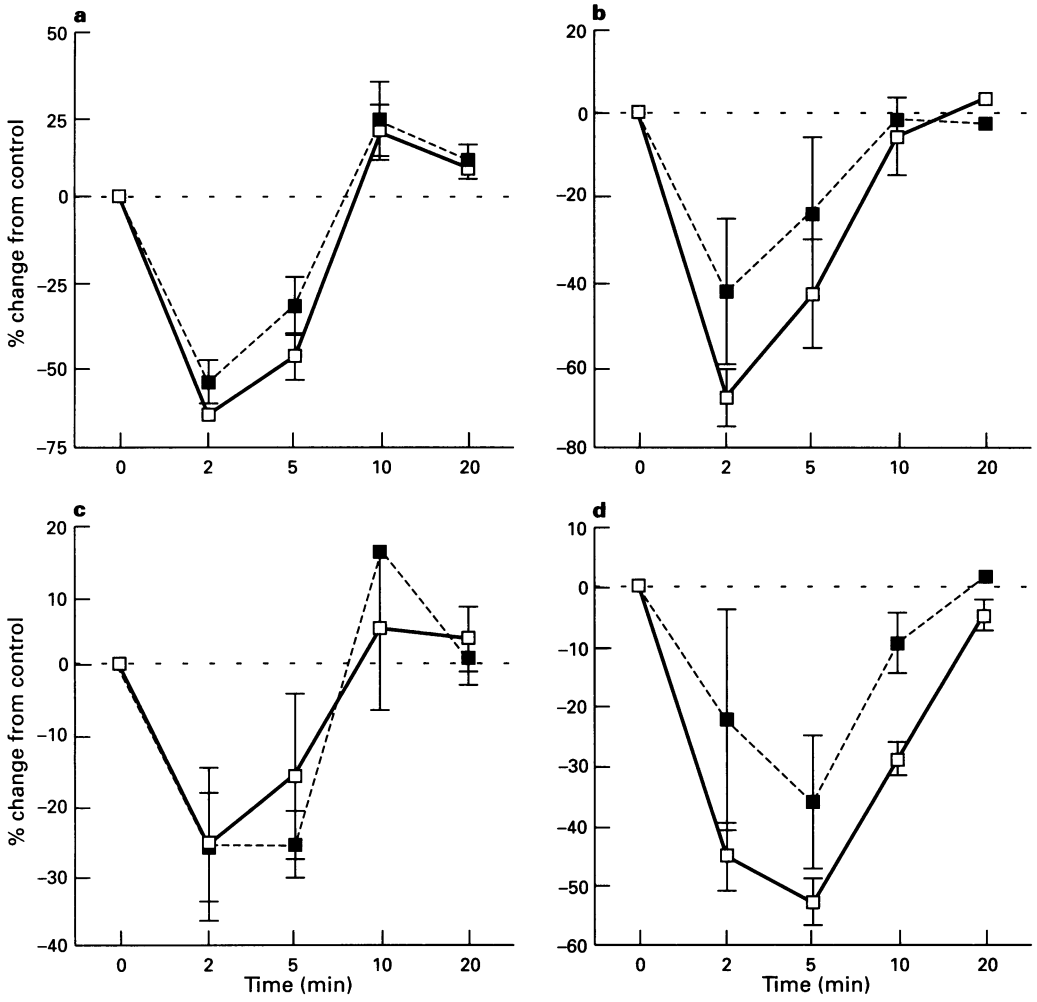

Figure 5 Effects of inhalation exposure of conscious OA-sensitized guinea-pigs upon the bronchoconstrictor responses to 50 μg ml^{-1} 5-HT (a, $n=8$), 100 μg ml^{-1} methacholine (MCh, b, $n=4$), 0.03 μg ml^{-1} U-46619 (c, $n=6$) and 1 mg ml^{-1} adenosine (d, $n=4$). Animals were exposed to the inhalations of 5-HT, MCh, or U-46619 at 24 h or adenosine at 7 days before saline challenge (□) and again at 18–24 h after the challenge (■). Changes in sG_{aw} values are expressed as mean ± s.e.mean percentage change from the pre-inhalation basal value.

by 1 mg and 10 μg kg^{-1} atropine was significant. However, comparison of the values before and after atropine at 2 and 5 min by ANOVA showed significant blockade of MCh and 5-HT ($P<0.05$) in all cases (Table 1).

Discussion

This study has demonstrated that exposure of conscious, sensitized guinea-pigs to an OA inhalation challenge produces a significant reduction in the bronchoconstriction to 5-HT or MCh at 17–24 h afterwards, compared with the exposure 24 h beforehand i.e. hyporeactivity. This was an unexpected finding. Hyperreactivity might have been anticipated since previous studies have demonstrated increased reactivity to 5-HT and ACh in anaesthetized guinea-pigs following bronchial anaphylaxis (Daffonchio *et al.*, 1987; 1989; Coyle *et al.*, 1988; Masaki *et al.*, 1994). Initially it was thought that the doses of 5-HT or MCh were too large and produced near maximal bronchoconstriction so that no further increase in this constriction could be seen. However, when a lower threshold dose of 5-HT was used for the bronchoconstriction, hyperreactivity again was not observed and the OA challenge appeared to inhibit the response to 5-HT 24 h later.

Hyperreactivity to 5-HT, MCh and acetylcholine (ACh) has repeatedly been demonstrated in anaesthetized guinea-pig models. Daffonchio *et al.* (1987; 1988; 1989) have demonstrated non-specific and route-independent bronchial hyperreactivity and hyperresponsiveness to i.v. and aerosol challenges of 5-HT and ACh in antigen-sensitized and challenged guinea-pigs. They concluded that the early release of endogenous histamine during bronchial anaphylaxis may modulate the release of as yet unidentified secondary mediators of airway hyperreactivity from resident pulmonary leucocytes. However, it has been shown in this laboratory that the demonstration of hyperreactivity (i.e. a leftward shift in the dose-response curve) or hyperresponsiveness (i.e. an increase in the maximum response) will vary depending on whether a full dose-response curve is performed or only a few doses on the curve are used (Johnson & Broadley, 1995).

Various theories have been put forward to explain airway hyperreactivity. Sanjar *et al.* (1990) have shown airway reactivity to histamine and $PGF_{2\alpha}$ in anaesthetized guinea-pig models whilst investigating the association between pulmonary airway eosinophilia and acute airway hyperreactivity. They conclude that eosinophilia may occur in association with increased airway reactivity, but there is no evidence for a causal relationship. Airway responsiveness to ACh in anaesthetized guinea-pigs sensitized to OA by inhalation, has been related, in part, to the altered airway epithelial functions (Masaki *et al.*, 1994). PAF has been suggested to play a central role in the antigen-induced eosinophil accumulation and subsequent bronchial hyperreactivity to i.v. ACh or histamine (Coyle *et al.*, 1988). Similarly, bronchial hyperresponsiveness has been

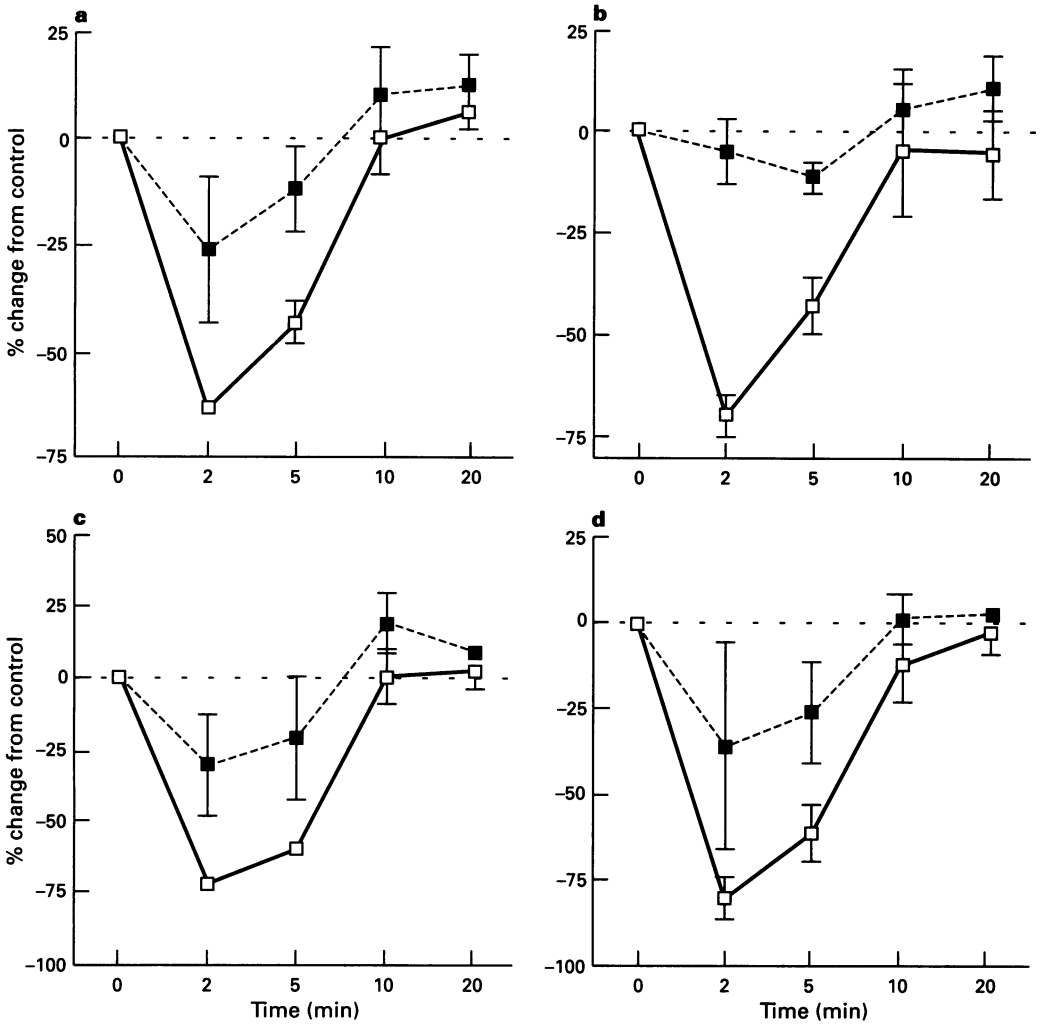

Figure 6 Effects of atropine (100 μg kg^{-1} left panels or 1 mg kg^{-1} i.p. right panels) on the bronchoconstriction of sensitized conscious guinea-pigs induced by 50 μg ml^{-1} 5-HT (a and c) and 300 μg ml^{-1} methacholine (b and d). Animals were exposed to inhalations of 5-HT or methacholine 24 h before atropine (□) and again at 0.5 to 1.5 h afterwards (■). Changes in sG_{aw} values are expressed as the mean ± s.e.mean ($n = 4$) percentage change from the pre-inhalation basal value.

Table 1 Blockade of the 5-HT- and methacholine-induced bronchoconstriction by atropine in conscious guinea-pigs

		% change in sG_{aw} from basal value at 2 min				
Spasmogen	*Atropine dose*	*before atropine*	*after atropine*	*% inhibition by atropine*	n	P *value*
5-HT	1 mg kg^{-1}	−69.74 ± 5.32	−5.09 ± 7.81*	88.0 ± 12.1	4	0.000025
(50 μg ml^{-1})	100 μg kg^{-1}	−62.91 ± 5.06	−26.06 ± 17.13	57.4 ± 27.7	4	0.007
	10 μg kg^{-1}	−75.35 ± 7.65	−47.13 ± 4.38*	53.6 ± 7.5	4	0.00069
MCh	1 mg kg^{-1}	−80.13 ± 6.24	−35.73 ± 29.77	69.9 ± 31.4	4	0.032
(300 μg ml^{-1})	100 μg kg^{-1}	−75.28 ± 2.98	−30.26 ± 17.64	56.6 ± 24.7	4	0.008

Peak bronchoconstriction by 5-HT or MCh was measured as the change from baseline of specific airways conductance (sG_{aw}) at 2 min. To determine the degree of inhibition by atropine, the values after atropine injection were expressed as a percentage of the pre-atropine values and the % values were subtracted from 100. *Significantly different from the pre-atropine change in sG_{aw} value ($P < 0.05$), determined by Student's paired t test. The P value was determined by Analysis of Variance (single factor) for the changes in sG_{aw} at 2 and 5 min, where $P < 0.05$ was considered significant.

demonstrated in actively sensitized and anaesthetized Brown Norway rats to i.v. 5-HT or MCh (Kips *et al.*, 1992; Wang *et al.*, 1993).

Previous studies in conscious, sensitized guinea-pigs have also demonstrated hyperreactivity to inhalations of histamine 1 h (Griffiths-Johnson *et al.*, 1991) and 3 and 6 h (Tarayre *et al.*, 1990) following an aerosolized challenge of ovalbumin. Featherstone *et al.* (1988) demonstrated increased reactivity to inhaled histamine and methacholine in guinea-pigs sensitized to ovalbumin by two inhalation challenges one week apart. In contrast, the present series of experiments have shown that the ovalbumin challenge rather than causing hyperreactivity causes an inhibition of the response to the spasmogen. It has been suggested that axon (local) reflexes induced by inflammatory mediators such as bradykinin on exposed C-fibre afferent nerve endings caused by damage to airway epithelium, could account

for at least some of the pathophysiology of asthma (Barnes, 1986). This would subsequently release sensory neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide which are potent inducers of airway smooth muscle contraction, bronchial oedema, extravasation of plasma, mucus hypersecretion, and possibly inflammatory cell infiltration and secretion (Barnes, 1986). Matsuse *et al.* (1991) recently reported that an OA challenge fails to induce bronchoconstrictor hyperresponsiveness in sensitized animals in which tachykinins (i.e. sensory neuropeptides), had been depleted with capsaicin. This result implies that tachykinins are released on OA challenge and may be involved in the hyperreactivity. The precise rate of synthetic replenishment after tachykinin depletion is unknown. This theory is supported by Hsiue *et al.* (1993) who demonstrated that reduced endogenous tachykinin release (due to impaired release or reduced storage granule content) after sensory nerve stimulation by dry gas hyperpnoea or by i.v. capsaicin administration would oppose the end-organ constrictor hyperresponsiveness in anaesthetized guinea-pigs, confirmed by exogenous i.v. ACh and i.v. tachykinin administration. They observed apparently normal constrictor responses to sensory C-fibre stimulation in OA-sensitized and challenged guinea-pigs. It is possible that 5-HT and MCh were acting locally via a sensory neural pathway in the present experiments and that OA challenge had depleted sensory neurones of endogenous tachykinins, thus providing a possible explanation for the apparent hyporeactivity. Furthermore, this may be a phenomenon specific to conscious guinea-pigs since hyporeactivity has not been observed in anaesthetized guinea-pigs, sensitized and challenged with OA using an identical protocol, in these laboratories (Johnson & Broadley, 1995).

However, the present experiments have also demonstrated a dose-related blockade by atropine of the 5-HT- and MCh-induced bronchoconstriction over the same antagonist dose-range. This suggests that the bronchoconstriction by 5-HT is mediated via muscarinic receptors, possibly by stimulating the afferent pathways of a vagal reflex. Therefore, in a conscious model 5-HT and MCh may act via a local neural pathway or via a vagal reflex or by a combination of both mechanisms. In anaesthetized animals, the bronchoconstriction by 5-HT may be mediated by a direct effect on airway smooth muscle 5-HT receptors. Hahn *et al.* (1978) however, demonstrated that 5-HT acts to potentiate vagal effects on airway smooth muscle in the lung of anaesthetized dogs, via the efferent vagal pathway.

An alternative explanation for the apparent hyporeactivity may be an increased mucus production, oedema and bronchial wall thickening in allergic airway inflammation which would prevent 5-HT and MCh from reaching their sites of action after inhalation. However, it has been shown in this laboratory (unpublished observation) that there is no difference in the wet and dry weight ratio of lungs taken from OA- and saline-challenged, sensitized guinea-pigs. Also morphometric analysis of airways from guinea-pigs given repeated allergen challenge by previous authors, showed no increase in epithelial or airway wall area (Ishida *et al.*, 1989).

In contrast to the effects of OA challenge on 5-HT- and MCh-induced bronchoconstriction, exposure to the thromboxane-mimetic, U-46619, demonstrated a significant increase in airway hyperresponsiveness 17–24 h after an OA challenge compared with the exposure beforehand. U-46619 is thought to have primarily a direct effect on thromboxane receptors of airway smooth muscle and a minor indirect effect via acetylcholine release (Jones *et al.*, 1992). It is the former which probably allows the appearance of airway hyperreactivity in the present study. U-46619 has been shown in human subjects to be a potent bronchoconstrictor and can cause airway hyperresponsiveness to inhaled methacholine in asthmatic subjects (Jones *et al.*, 1992). Similarly, thromboxane-A_2 (TxA_2) has been shown in guinea-pig models using ozone (Daniel & O'Byrne, 1991) and antigen challenge (Arimura *et al.*, 1994) to contribute to airway bronchoconstriction and underlying bronchial hyperresponsiveness. It is not thought that the hyperresponsiveness observed is due to augmentation of endogenous TxA_2 release by the first challenge with U-46619 since the U-46619 challenge was given 17–24 h after the OA exposure when endogenous levels of TxA_2 should have reduced.

Inhaled adenosine produced a bronchoconstriction in sensitized guinea-pigs. This confirms previous observations using adenosine in our laboratories (Thorne & Broadley, 1994). It is well known that asthmatic subjects respond to adenosine (or 5'-AMP) with a bronchoconstriction whereas non-asthmatics display no response (Cushey *et al.*, 1984; Mann *et al.*, 1986; Broadley, 1995). Isolated bronchi from asthmatic subjects show a contractile response which is thought to be mediated indirectly by liberation of leukotrienes and histamine (Björck *et al.*, 1992). Similarly, isolated lung tissues from sensitized guinea-pigs show a constrictor response to adenosine whereas tissues from unsensitized animals display the more usual direct smooth muscle relaxant effects mediated via A_2 receptors (Thorne & Broadley, 1992). The bronchoconstrictor responses to adenosine or 5'-AMP in whole animals (Thorne & Broadley, 1994) human subjects (Phillips *et al.*, 1989) and isolated tissues (Thorne & Broadley, 1992) are rapidly tachyphylactic. Thus, in the present experiments the exposures to adenosine were placed 8 days apart, (the ovalbumin exposure being given on day 7). This resulted in reproducible bronchoconstrictions. The bronchoconstrictions induced by 5-HT, MCh and U-46619 were also reproducible when given only 2 days apart. At 18–24 h after OA inhalation, the bronchoconstriction by adenosine, however, was neither enhanced nor inhibited. It is possible that the contraction was near maximal and therefore potentiation was not possible. The lack of inhibition suggests that adenosine does not mediate bronchoconstriction via the OA-sensitive routes used by 5-HT and MCh.

In summary, U-46619 was the only spasmogen used which demonstrated airway hyperresponsiveness *in vivo* at 24 h after OA inhalation of conscious, OA-sensitized guinea-pigs. Ovalbumin challenge inhibited the bronchoconstriction to 5-HT and MCh and may therefore interfere with an indirect component of the responses to these spasmogens. U-46619 presumably acts directly on smooth muscle and explains why hyperreactivity was observed. Furthermore, adenosine was shown to cause bronchoconstriction in sensitized guinea-pigs but hyperresponsiveness was not observed with the spasmogen after an OA challenge.

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