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Immediate anaphylactic bronchoconstriction induces airway hyperreactivity in anaesthetized guinea-pigs

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1 The possible acute occurrence of airway hyperreactivity after immediate-type bronchial anaphylaxis has been investigated in anaesthetized guinea-pigs actively sensitized to ovalbumin (OA).

2 Aerosol challenge (OA 10 mg ml^{-1}, 5 s) provoked immediate bronchoconstriction which was substantially, although incompletely, reversed by isoprenaline (Iso) infusion (1 μg kg^{-1} min^{-1}) for 10 min).

3 Bronchoconstrictor responses to 5-hydroxytryptamine (5-HT) were enhanced in challenged animals when compared to those in non-challenged animals that had also received Iso. This was seen as a leftward shift in the location of the dose-response curve for the bronchoconstrictor effect of 5-HT (dose-ratio 2.45, 95% confidence limits 1.77–3.38; $P < 0.01$). This phenomenon was associated with pulmonary infiltration of polymorphonuclear leukocytes, which was not modified by Iso treatment.

4 Iso infusion alone caused a slight enhancement of airway reactivity seen as a small leftward shift of the dose-response curve for the bronchoconstrictor effect of 5-HT (dose-ratio 1.51, 95% confidence limits 1.07–2.13; $P < 0.05$).

5 These results support a causal relationship between acute pulmonary inflammation and airway hyperreactivity in an animal model of human allergic asthma.

Introduction

A non specific enhancement of bronchial smooth muscle responsiveness (airway hyperreactivity) to pharmacological and environmental stimuli, is one of the major features of asthma (Boushey *et al.*, 1980). Clinical studies have more commonly linked this phenomenon to the late, rather than early, asthmatic response to antigen-provocation (Cockroft *et al.*, 1977; Cartier *et al.*, 1982). Furthermore, airway hyperreactivity has been similarly demonstrated in different animal models following a late 'asthmatic' reaction, and in parallel with polymorphonuclear leukocyte (PMN) recruitment (Marsh *et al.*, 1985; Murphy *et al.*, 1986). Airway hyperreactivity has also been shown to be associated with pulmonary inflammation induced by non allergic stimuli (Empey *et al.*, 1976; Fabbri, *et al.*, 1984). Accordingly, the possible underlying role of inflammatory cells in the genesis of airway hyperreactivity has been recognised, and may represent a common pathological event in airway hyperreactivity irrespective of its origin (O'Byrne, 1986).

Ovalbumin sensitized guinea-pigs are widely used as animal models of asthma. In this species, conventional (i.v. and/or s.c.) sensitization and antigen challenge procedures (e.g. i.v.) lead to a severe immediate anaphylactic bronchoconstriction which is not usually followed by a late response. However, exogenous administration of different anaphylactic mediators has been demonstrated to induce airway hyperreactivity in guinea-pigs both *in vitro* and *in vivo* (Creese & Bach, 1983; Thorpe & Murlas, 1986; Omini *et al.*, 1986). Challenge of sensitized guinea-pigs with antigen aerosol results in inflammatory sequelae similar to that described in asthmatic subjects (Kallos & Kallos, 1984). Hence, in the present study, we investigated the possible occurrence of airway hyperreactivity after acute bronchial anaphylaxis induced in anaesthetized, sensitized, guinea-pigs by a newly developed technique for challenge with antigen aerosol (Payne & Nucci, 1987). This method

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has recently been characterized pharmacologically (Daffonchio *et al.*, 1987a). Some of these results have been communciated to the British Pharmacological Society (Daffonchio *et al.*, 1987b).

Methods

Sensitization and challenge procedure

Male Dunkin Hartley guinea-pigs (300–350 g) were actively sensitized to ovalbumin (OA, sigma grade V, 50 mg i.p. plus 50 mg s.c.). After 14 to 21 days, animals were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), both cervical vagi were cut, and the trachea cannulated. Animals were thereafter mechanically ventilated by a Searle BioScience constant volume respiratory pump delivering 54 strokes min^{-1} of 1 ml of air per 100 g body weight. Pulmonary inflation pressure (PIP), an index of intrathoracic airway calibre, was measured in cmH_2O from a lateral port in the afferent limb of the ventilator circuit, with a pressure transducer (Statham Pb 21AA) connected to a Grass model 7D polygraph. Basal PIP ranged from 8 to 15 cmH_2O and was not statistically different in the various treatment groups considered. A jugular vein was cannulated for drug administration. After a 10 min stabilisation period, animals were challenged for 5 s with an aqueous aerosol of OA, 10 mg ml^{-1} in saline, generated by a DeVilbiss 'Pulmosonic' Ultrasonic nebuliser (Payne & Nucci, 1987). As the resulting anaphylactic bronchoconstriction was to a large degree not spontaneously reversible, isoprenaline (Iso, 1 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) was infused for 10 min, (starting 10 min after) OA challenge in order to reduce substantially the remaining rise in PIP. Control groups of animals were incorporated in the study as detailed below.

Functional studies

The functional development of airway hyperreactivity after antigen challenge, assessed as changes in the bronchoconstrictor effect of 5-HT, was evaluated in three groups of sensitized guinea-pigs: These were (a) non-challenged (absolute controls), (b) non-challenged + Iso-treated (Iso – controls) and (c) challenged + Iso animals. It is important to note that dose-response curves for the bronchoconstrictor effect of 5-HT (1–30 $\mu g\,kg^{-1}$, i.v.) were constructed subsequently 40 min after cessation of Iso infusion (1 h after antigen exposure in challenged animals) and at the same time interval after initial stabilisation in the absolute controls.

Histological studies

Histological studies were performed in challenged animals and in some animals after Iso infusion according to the experimental procedure described above. At 10, 20 and 60 min after OA challenge, representative animals (minimum of $n = 3$ per group) were killed after first administering heparin (100–150 units i.v.). The lungs were removed, inflated with 10 ml of air and perfused via the pulmonary artery with 25 ml of phosphate buffered saline (pH 7.2) at a constant flow rate of 5 ml min^{-1}. Modified Karnovskys fixative (5 ml) was injected via the trachea and an additional 25 ml was infused via the pulmonary artery at 5 ml min^{-1}. The trachea was then ligated and the lungs immersed in fixative and stored at +4°C. Caudal lobes were later processed for wax histology and 4 μm sections cut and stained with haematoxylin and eosin for examination by light microscopy.

Drugs

The following drugs were used: sodium pentobarbitone (BDH); 5-HT, (−)-isoprenaline sulphate, aminophylline, (Sigma). Sodium pentobarbitone was supplied as a solution of 60 mg ml^{-1} and was diluted to 10 mg ml^{-1} with 0.9% w/v NaCl solution (saline). Fresh stock solutions of the other drugs were also prepared daily in saline.

Statistical analysis

Data are expressed as the mean ± s.e. of (n) experiments. Dose-response curves to 5-HT in different experimental groups were compared by means of variance analysis according to Finney's biological assay procedure (Finney, 1952) and the dose-ratio (DR) with 95% confidence limits calculated.

Results

Challenge with OA aerosol provoked immediate bronchoconstriction measured as a sustained rise in PIP (maximum increase 46.8 ± 2.7 cmH_2O at 2 min, $n = 6$.) Iso infusion (1 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) substantially reduced the degree of residual bronchoconstriction still remaining at 10 min post-challenge (Figure 1). This reduction was however, incomplete and an increased PIP of 10.3 ± 1.3 cmH_2O, ($n = 6$), was still present at the end of Iso administration; this new basal value was maintained throughout the remaining part of the experiment. Bolus injections of high doses of Iso, (up to 100 $\mu g\,kg^{-1}$, i.v.), or aminophylline, 10 mg kg^{-1}, i.v.), did not significantly reduce this baseline further (3 experiments for each).

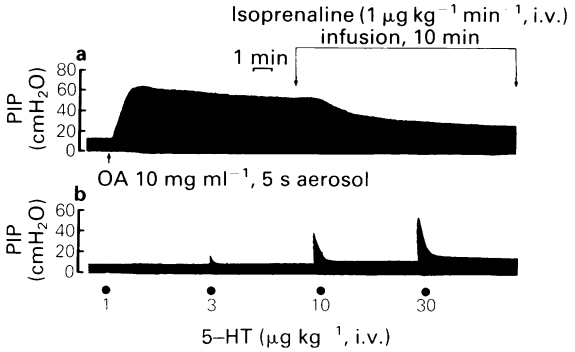

Figure 1 (a) Original tracing from a typical experiment in an anaesthetized sensitized guinea-pig showing the sustained increase in pulmonary inflation pressure (PIP) provoked by aerosol challenge (OA, 10 mg ml^{-1}, 5 s) and its substantial reversal by isoprenaline (Iso) infusion (1 μg kg^{-1} min^{-1}, i.v. × 10 min). (b) Original tracing from a typical experiment in an anaesthetized sensitized guinea-pig (non-challenged absolute control) showing the rise in PIP provoked by sequential administration of increasing doses of 5-hydroxytryptamine (5-HT) i.v.

Anaphylactic bronchospasm was followed by the subsequent development of airway hyperreactivity as indicated by a potentiation of the bronchoconstrictor effect of 5-HT in those animals challenged. Dose-response curves to 5-HT (1–30 μg kg^{-1}, i.v.) obtained 1 h after OA exposure were significantly shifted to the left ($P < 0.01$) as compared to unchallenged Iso control animals (Figure 2). In this respect the DR was 2.45 (95% confidence limits 1.77–3.38). This effect only occurred after antigen challenge since in a separate series of experiments saline aerosol for 5 s did not significantly modify either basal PIP (pre 13.9 ± 0.9 cmH_2O, post 14.6 ± 1.2 cmH_2O, $n = 6$; $P > 0.05$) or the bronchoconstrictor effect of 5-HT. By reference to results obtained previously in non-sensitized guinea-pigs we found no evidence to suggest that the sensitization protocol used in the present study in itself caused an increase in airway reactivity to 5-HT. However, on detailed analysis of data it became clear that Iso treatment alone slightly potentiated 5-HT-induced bronchoconstriction as shown in Figure 3. Dose-response curves for the bronchoconstrictor effect of

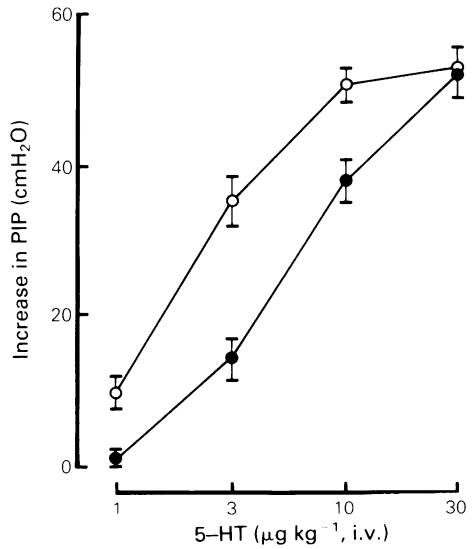

Figure 2 Dose-response curves constructed sequentially for the increase in pulmonary inflation pressure (PIP) induced by 5-hydroxytryptamine (5-HT) i.v. in anaesthetized sensitized guinea-pigs: (●) isoprenaline (Iso) control group (non challenged + Iso infusion 1 μg kg^{-1} min^{-1} i.v. × 10 min; $n = 6$); (○) challenged group (OA aerosol 10 mg ml^{-1}, 5 s, + Iso infusion 1 μg kg^{-1} min^{-1} i.v. × 10 min; $n = 6$). Points show the mean results ± s.e. mean (vertical lines) obtained at each 5-HT dose level. The dose-response curve for 5-HT was significantly ($P < 0.01$) shifted to the left in those animals challenged with OA (DR = 2.45, 95% confidence limits 1.77–3.38).

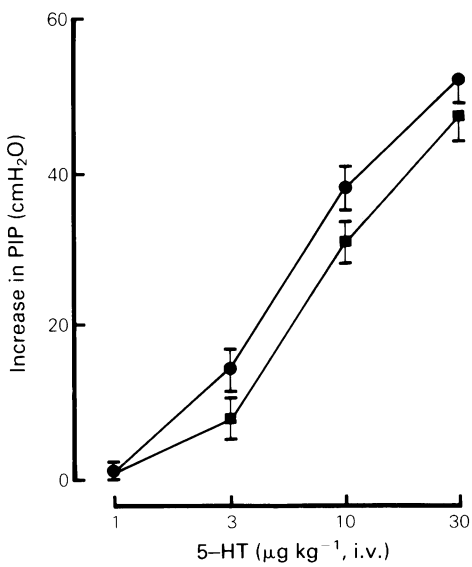

Figure 3 Effect of prior infusion of isoprenaline (Iso; 1 μg kg^{-1} kg^{-1} min^{-1}, i.v. × 10 min) on the dose-response curve for the increase in pulmonary inflation pressure (PIP) induced by 5-hydroxytryptamine (5-HT) i.v. in anaesthetized sensitized guinea-pigs: (■) non challenged (absolute control) animals, $n = 6$; (●) non challenged + Iso (Iso control) animals, $n = 6$. Points shown indicate the mean result ± s.e. mean (vertical lines) obtained at each 5-HT dose level. The dose-response curve for 5-HT was significantly ($P < 0.05$) shifted to the left in those animals that had received Iso (DR = 1.51, 95% confidence limits 1.07–2.13).

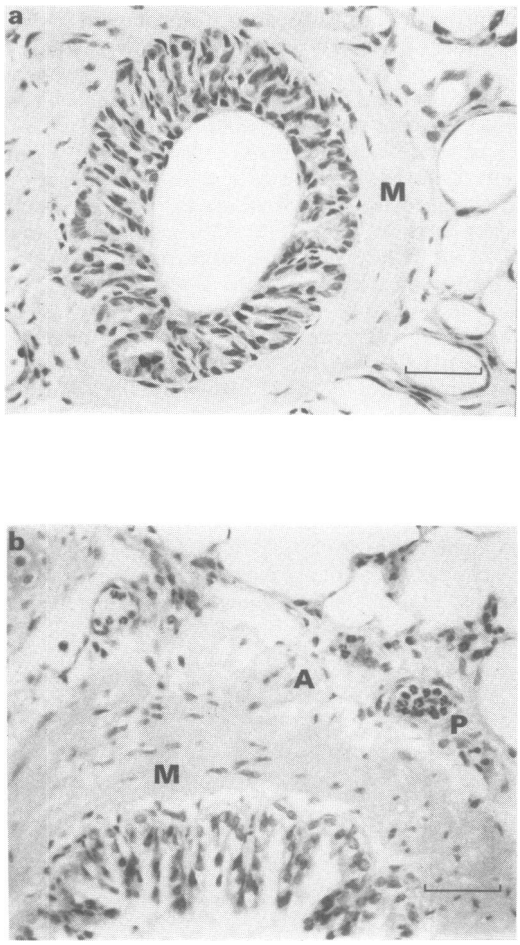

Figure 4 Photomicrographs of 4 μm haematoxylin and eosin stained sections of airways within the caudal lobe of lungs of (a) a sensitized, unchallenged guinea pig and (b) a sensitized guinea-pig killed 20 min after aerosol challenge (OA, 10 mg ml^{-1}, 5 s). The respiratory epithelium is highly folded as a result of smooth muscle (M) contraction in both samples, but otherwise appears normal. The adventitia (A) in the challenged lung is oedematous and polymorphonuclear leukocytes (P) can be seen in the blood vessels. The solid horizontal bar in the bottom right of each photomicrograph indicates a width of 0.5 mm.

5-HT obtained 40 min after Iso infusion alone (no challenge) were statistically shifted to the left ($P < 0.05$) of those obtained in absolute control (sensitized, non-challenged) guinea-pigs that had not received Iso. The DR was 1.51 (95% confidence limits 1.07–2.13).

Histological analysis of lungs removed from challenged animals showed that the immediate anaphylactic bronchoconstriction was accompanied by an inflammatory reaction within the airways. Recruitment of PMNs was observed, mainly in the bronchial adventitia, but also in the vasculature, as early as 10 min after OA administration, and more obviously 20 min post-challenge (Figure 4). PMN infiltration was not reversed by the subsequent Iso treatment. Moreover, the presence of these inflammatory cells was still evident 1 h after antigen challenge at which time hyperreactivity was assessed. In contrast, vascular margination of PMNs within blood vessels seemed to be a more transient phenomenon. It was appreciable up to 20 min, with or without Iso perfusion. However, no PMNs adhering to the vessels were observed 1 h after antigen challenge. Evidence of generalised and, more particularly, lymphatic oedema was also seen following antigen challenge, and in one instance the occurence of haemorrhagic foci was noted in the lung parenchyma.

Discussion

Airway hyperreactivity is an important feature of asthma on which much current attention is being focussed. Several reports suggest that products of arachidonic acid metabolism, from both the cyclooxygenase and lipoxygenase pathways (Robinson & Holgate, 1985), might play a role in this phenomenon. In addition, platelet-activating factor (Paf-acether) has recently been shown to potentiate methacholine-induced bronchoconstriction in man (Cuss *et al.*, 1986) adding to the list of possible mediators of airway-hyperreactivity.

Regardless of which mediators might be relevant to this phenomenon, an important question is whether their release during bronchial anaphylaxis *in vivo*, could acutely induce airway hyperreactivity. Our results clearly show that in anaesthetized guinea-pigs, anaphylactic mediators released during an immediate type antigen-induced bronchoconstriction can indeed cause acute bronchial hyperresponsiveness as indicated by potentiation of the bronchoconstrictor effect of 5-HT. We have additionally shown in a preliminary report, that airway hyperreactivity induced in guinea-pigs by a challenge with antigen aerosol is not specific to 5-HT but extends also to acetylcholine (Daffonchio *et al.*, 1987b). Furthermore, as this phenomena occurs after an 'immediate type' response, this shows that in this species a late 'asthmatic' response is not required for development of airway hyperreactivity. These findings are in agreement with the results of Hogg *et al.*, (1985) showing that in allergic sheep the respiratory

expression of the late response is not obligatory for subsequent airway hyperreactivity to occur. A clinical parallel is that in man, sodium cromoglycate can prevent allergen-induced bronchial hyperresponsiveness at doses that do not alter the magnitude of the late asthmatic response (Mattoli *et al.*, 1987).

An association between the development of bronchial hyperresponsiveness and the recruitment of inflammatory cells into the lung has been observed in both allergic and non-allergic induced airway hyperreactivity (Fabbri *et al.*, 1984; Hogg *et al.*, 1985; Murphy *et al.*, 1986). Our data support such a relationship since both PMN infiltration and pulmonary hyperresponsiveness were observed concomitantly within 60 min of antigen challenge. However, these data do not prove a causal association between these two events and it is possible that although occurring concurrently they may be independent phenomena. In this regard, it has recently been shown that PMN depletion by two different agents does not affect toluene di-isocyanate-induced bronchial hyperresponsiveness in guinea-pigs. (Thompson *et al.*, 1986), suggesting that in this instance the presence of inflammatory cells is not a prerequisite for the genesis of airway hyperreactivity. On the other hand, we feel that it is important to emphasize that Iso treatment did not modify either PMN recruitment nor did it prevent the enhancement of 5-HT-induced bronchoconstriction following the anaphylactic reaction. This latter observation concurs with the inability of β-adrenoceptor agonists to prevent bronchial hyperresponsiveness in man (Cockcroft & Murdock, 1987) and indirectly supports a correlation between inflammatory (rather than contractile) processes within the lung and the development of airway hyperreactivity.

In non-challenged animals, Iso infusion alone slightly potentiated subsequent 5-HT-induced bronchoconstriction. Other studies have reported enhancement of histamine-induced bronchospasm and increased mortality after prolonged treatment with β-adrenoceptor agonists (Conolly *et al.*, 1971; Williams *et al.*, 1983). A possible common underlying mechanism is desensitization of β-adrenoceptors. As a consequence the bronchodilator effect of intrinsic neuronal and hormonal influences may be reduced. Arguing against this proposition, Mazzoni *et al.*, (1987) have recently reported that, in guinea-pigs, Iso causes airway hyperreactivity by a mechanism unrelated to β-adrenoceptor occupancy. However, irrespective of the actual mechanism involved, in the present study the degree of hyperreactivity induced by Iso alone was clearly less than that seen in animals challenged with antigen.

It is possible that β-adrenoceptor desensitization explains the reduced bronchodilator effect of Iso after antigen challenge. Indeed, quantitative changes in pulmonary β-adrenoceptor number have been detected following repeated antigen-challenge in sensitized guinea-pigs (Gatto *et al.*, 1987). Iso also fails to reverse completely airways obstruction in guinea-pigs rendered hyperreactive by prior exposure to Paf-acether (Barnes *et al.*, 1987), which is a putative mediator of anaphylactic bronchospasm in this species (Braquet *et al.*, 1985; Casals-Stenzel, 1987). There is however, no alteration in either β-adrenoceptor number or function in pulmonary tissue excised from animals exposed to Paf-acether (Barnes *et al.*, 1987). Furthermore in the present study the phosphodiesterase inhibitor aminophylline was also ineffective in reversing completely anaphylactic bronchoconstriction. Thus whilst it cannot be totally discounted that anaphylactic mediators other than Paf-acether may influence β-adrenoceptor function acutely, an alternative explanation for the reduced bronchodilator effect of Iso (and aminophylline) after antigen challenge is the involvement of non-contractile elements, e.g. oedema, mucous plugging and small airway collapse.

Owing to the fourth power relationship between airway radius and resistance to air flow it could be argued that the residual increase in PIP following antigen challenge is responsible for the subsequent increase in airway reactivity. However, whilst this may be a contributing factor, we think that it is unlikely to be solely responsible as anaphylactic microshock, which results in a significantly ($P < 0.05$) smaller residual rise in PIP, causes a similar degree of airway hyperreactivity, (Daffonchio *et al.*, 1987b). Also clinical studies have shown that in normal subjects bronchoconstriction with methacholine does not influence significantly the concomitant bronchoconstrictor effect of histamine (Chung & Snashell, 1984).

In conclusion, our data show that in guinea-pigs, the endogenous release of anaphylactic mediators provoked by a single aerosol challenge, can induce acutely airway hyperreactivity against a background of an inflammatory lesion. Which mediator or mediators might be responsible remains to be established. Such an acute phenomenon might be, in particular conditions, a possible cause of the late asthmatic reaction in man rather than its consequence. The recent finding that bronchial reactivity increases soon after the immediate response in dual-responding asthmatic subjects (Thorpe *et al.*, 1987) supports such a hypothesis.

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