

Effects of endotoxin and allergen alone and in combination on the sensitivity of the rat airways to adenosine

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Summary

1 The airways of patients with asthma are hyperresponsive to adenosine. The phenomenon can be mimicked in the actively sensitized Brown Norway rat by exposure to allergen or lipopolysaccharide (LPS). We wondered whether combined treatment with allergen and endotoxin would result in additive effects or synergism with respect to increasing the sensitivity of the airways of the Brown Norway rat to adenosine.

2 Animals actively sensitized to ovalbumin and challenged intratracheally with allergen or endotoxin manifested increased bronchoconstrictor responses to adenosine. A combination of ovalbumin and endotoxin also increased the response to adenosine but the effects were at best additive.

3 Changes in the response to adenosine were selective as responses to 5-hydroxytryptamine were unaltered following ovalbumin or LPS either alone or in combination.

4 Thus, endotoxin and allergen acting together could play a role in up-regulating the response of the human asthmatic airway to adenosine. However, our data suggest that the interaction would be additive rather than synergistic.

Keywords: adenosine, airway hyperresponsiveness, allergen challenge, ovalbumin, endotoxin, Brown Norway rat

Introduction

The responsiveness of the airways of the Brown Norway rat to adenosine is markedly increased in actively sensitized animals challenged with ovalbumin (Hannon, Tigani, Williams, Mazzoni & Fozard, 2001; Hannon *et al.*, 2002; Fozard *et al.*, 2003). The augmented response to adenosine is mast cell mediated and in this respect bears similarities to the bronchoconstrictor response to inhaled adenosine which is markedly up-regulated in asthmatic patients (Fozard & Hannon, 2000; Meade, Dumont & Worrall, 2001; Van Den Berge, Polosa, Kerstjens & Postma, 2004). A similar selective augmentation of the bronchoconstrictor response to adenosine can be induced in non-sensitized Brown Norway rats by challenge with endotoxin (lipopolysaccharide, LPS) and again the mast cell is implicated in the response (Tigani, Hannon, Rondeau, Mazzoni & Fozard, 2002). While the A_{2B} receptor is believed to mediate the response to adenosine in asthmatics (Meade *et al.*, 2001; Fozard, 2003; Holgate, 2005), this subtype is

not involved in the augmented response to adenosine induced by LPS or allergen in the rat (Hannon *et al.*, 2002; Tigani *et al.*, 2002; Wolber & Fozard, 2005).

The mechanism of the up-regulation of the response to adenosine in the asthmatic airways is not understood. We questioned whether a combination of two quite different stimuli which when given independently enhance the airways to adenosine would act synergistically when given in combination. To this end, we carried out experiments to define the interaction between allergen and endotoxin in setting the sensitivity of the airways of the Brown Norway rat to adenosine.

Materials and methods

These have been described in detail recently (Ellis, Cannet, Mazzoni & Fozard, 2004). In brief, male Brown Norway rats weighing 200–300 g were actively sensitized to ovalbumin. For drug administration, animals were briefly anaesthetized with 4% isoflurane, and ovalbumin (10 or 30 µg kg⁻¹),

endotoxin (10 or 30 $\mu\text{g kg}^{-1}$) or the combination of ovalbumin and endotoxin (10 or 30 $\mu\text{g kg}^{-1}$) or the vehicle (saline, 0.2 ml per animal), was administered intratracheally (i.t.). The animals were then used in lung function experiments 1 h after the challenge.

Animals were anaesthetized with sodium pentothal (70 mg kg^{-1} , i.p.), treated intramuscularly with vecuronium bromide (12 mg kg^{-1}) and ventilated (7 ml kg^{-1} , 1 Hz) via the tracheal cannula with a mixture of air and oxygen (50:50, v/v). Airway resistance (R_L , $\text{cmH}_2\text{O l}^{-1} \text{ s}$) was calculated from measurements of airflow and transpulmonary pressure after each respiratory cycle by use of a digital electronic pulmonary monitoring system (Mumed, London, UK). Bronchoconstrictor responses to adenosine (1 mg kg^{-1} , i.v.) and 15 min later to 5-hydroxytryptamine (5-HT, 10 and 20 $\mu\text{g kg}^{-1}$) were established in all animals.

Pentothal (thiopentalum natricum) was obtained from Abbott (Baar, Switzerland). Norcuron (vecuronium bromide) was from Organon Teknika (Boxtel, the Netherlands). Ovalbumin was obtained from Fluka (Basel, Switzerland). LPS (from *Salmonella typhosa*), 5-HT creatinine sulphate and adenosine hemisulphate were obtained from Sigma (Basel, Switzerland). All compound were made up freshly in 0.9% (w/v) NaCl on the day of the experiment.

All data are presented as mean $\pm$ SEM. Statistical analysis was carried out on raw data using the

Student's *t*-test with the Bonferroni correction. A *P* value of <0.05 was considered significant.

Results

The data are presented in Figs 1 & 2. Responses to adenosine (1 mg kg^{-1} , i.v.) were significantly augmented 1 h following challenge with ovalbumin (10 and 30 $\mu\text{g kg}^{-1}$) or endotoxin (10 or 30 $\mu\text{g kg}^{-1}$). Following combined treatment with ovalbumin and endotoxin, each at 10 $\mu\text{g kg}^{-1}$, the bronchoconstrictor response to adenosine was no greater than that following ovalbumin alone and the combined effect was less than additive. When ovalbumin and endotoxin were combined, each at 30 $\mu\text{g kg}^{-1}$, the bronchoconstrictor response to adenosine was greater than the responses seen with either agent alone (significant for the 30 $\mu\text{g kg}^{-1}$ dose of ovalbumin) and the effect was additive. That the responses to adenosine and/or endotoxin were not curtailed due to a possible ceiling response in the system is indicated by the substantially greater response to a high dose of adenosine (3 mg kg^{-1}) in the presence of a high dose of ovalbumin (100 $\mu\text{g kg}^{-1}$) (Fig. 1). The sensitivity of the airways to 5-HT was not significantly altered by challenge with ovalbumin or endotoxin either alone or in combination (Fig. 2). The baseline resistance of the airways was not significantly altered following challenge with LPS, ovalbumin or the combination of both and was between 120 and 150 $\text{cmH}_2\text{O l}^{-1} \text{ s}$.

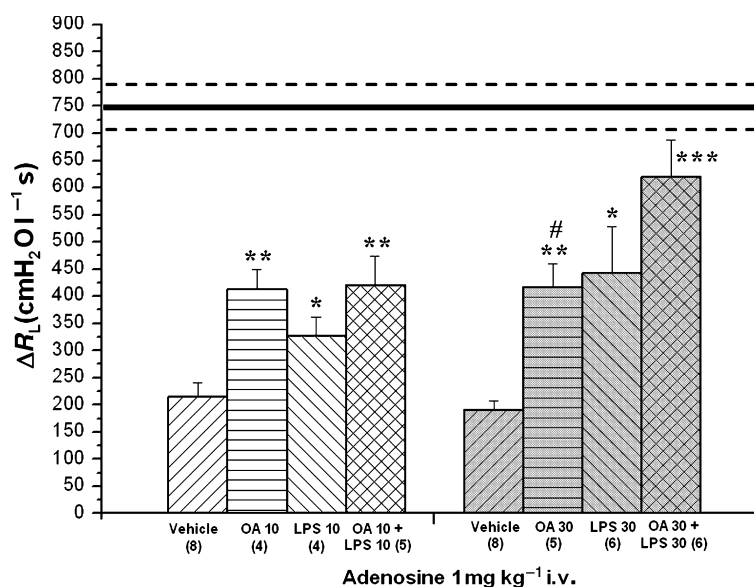


Figure 1 Effects of ovalbumin (OA) or endotoxin (LPS) alone or in combination on the bronchoconstrictor responses to adenosine (1 mg kg^{-1} , i.v.) elicited 1 h after treatment. Left histograms: ovalbumin and endotoxin, 10 $\mu\text{g kg}^{-1}$; right histograms ovalbumin and endotoxin, 30 $\mu\text{g kg}^{-1}$. Values are mean $\pm$ SEM of the numbers of individual observations shown in parentheses. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 that the value differs significantly from the equivalent value in the vehicle-treated group. #*P* < 0.05 that the value differs significantly from the value obtained with combined treatment. The horizontal line shows the mean response from four experiments to adenosine, 3 mg kg^{-1} i.v., 1 hr after administration of ovalbumin, 100 $\mu\text{g kg}^{-1}$, with the dashed line representing the SEM.

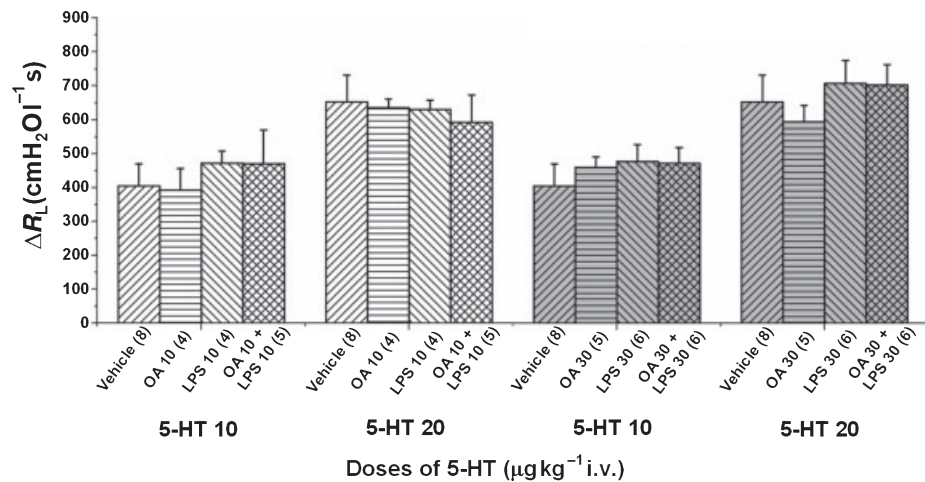


Figure 2 Effects of ovalbumin (OA) or endotoxin (LPS) alone or in combination on the bronchoconstrictor responses to 5-HT (10 and 20 µg kg⁻¹, i.v.) elicited 1 h after treatment. Left histograms: ovalbumin and endotoxin, 10 µg kg⁻¹; right histograms ovalbumin and endotoxin, 30 µg kg⁻¹. Values are mean ± SEM of the numbers of individual observations shown in parentheses.

Discussion

The evidence that adenosine is generated endogenously in asthma has been recently summarized (Fozard, 2003). Thus, adenosine production is increased by hypoxia and inflammatory cell activation. Bronchoalveolar lavage fluid and inhaled breath condensate concentrations of adenosine are elevated in asthmatics and plasma adenosine concentrations are increased by allergen challenge. Finally, adenosine concentrations in the plasma of asthmatics are increased by exercise and cross-tachyphylaxis occurs between AMP challenge and exercise-induced asthma.

We have confirmed that challenge of actively sensitized Brown Norway rats with ovalbumin rapidly induces airways hyperreactivity to adenosine (Hannon *et al.*, 2001) and that a similar effect can be seen following endotoxin (Tigani *et al.*, 2002). Our choice of 1 h for defining the interaction of ovalbumin and endotoxin with adenosine was dictated by the fact that in our earlier studies potentiation of adenosine by endotoxin was maximum at this time point (Tigani *et al.*, 2002). Our earlier studies also established that the response to adenosine augmented following ovalbumin or endotoxin was mast cell mediated (Hannon *et al.*, 2001; Tigani *et al.*, 2002).

The increased sensitivity to adenosine following ovalbumin stems from an increase in the reactivity of the mast cells of the lung induced by exposure to the allergen (Hannon *et al.*, 2001; Wolber, Baur & Fozard, 2004). The available evidence is consistent with a similar mechanism underlying the potentiation of adenosine by endotoxin (Tigani *et al.*, 2002). The mechanisms of mast cell activation by allergen and endotoxin are fundamentally different: the former involves cross linking of the high affinity FcεRI receptor by allergen (Siraganian, 2003) and

the latter acts through activation of Toll-like receptor 4 (McCurdy, Lin & Marshall, 2001). The possibility of a synergistic interaction arising from amplification of one intracellular signalling pathway by a separate coincident signal within a parallel but separate pathway could, therefore, be envisaged (Selbie & Hill, 1998). In the event, the present data show that combining the two pro-inflammatory stimuli induces an increase in response to adenosine but the effect is, at best, additive.

A variety of evidence implicates endotoxin as a contributory factor in human asthma (Liu, 2002). For example, asthmatic subjects are hypersensitive to endotoxin reacting with an acute and late phase bronchoconstrictor response (Michel, Duchateau & Sergysels, 1989). Moreover, low levels of endotoxin exposure significantly augment the inflammatory response to allergen both in atopic asthmatics (Hunt *et al.*, 1994) and actively sensitized Brown Norway rats (Tulic, Wale, Holt & Sly, 2000). We have speculated that endotoxin along with allergen could be a factor in determining the increased sensitivity of the airways of asthmatic patients to adenosine (Tigani *et al.*, 2002). That suggestion remains plausible; however, if the present data can be extrapolated to human, the effect would be additive to the effects of allergen rather than synergistic.

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