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Interaction between adenosine and allergen or compound 48/80 on lung parenchymal strips from actively sensitized Brown Norway rats

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Abstract

We have investigated the effect of mast cell activation induced by immunological and non-immunological stimuli on the sensitivity to adenosine of parenchymal strips prepared from lungs removed from Brown Norway (BN) rats actively sensitized to ovalbumin. Strips responded to ovalbumin with a biphasic contractile response. Responses to adenosine were markedly increased 30 min after ovalbumin. The first phase of the response to ovalbumin was abolished by the 5-hydroxytryptamine $(5\text{-HT})_2$ receptor antagonist, methysergide and unaffected by the cysteinyl leukotriene receptor antagonist, iralukast. The second phase was abolished by iralukast and unaffected by methysergide. The response to adenosine was markedly reduced by methysergide but not significantly altered by iralukast. Compound 48/80 (condensation product of *N*-methyl-*p*-methoxyphenylethylamine with formaldehyde) induced methysergide-sensitive contractions of the parenchymal strip and potentiated adenosine; the augmented response to adenosine was blocked by methysergide. Thus, activation of mast cells in the lung by either immunological or non-immunological stimuli results in augmentation of the mast cell-mediated contractile response to adenosine.

Keywords: Adenosine; Airway hyperresponsiveness; Allergen challenge; Ovalbumin; Compound 48/80; Rat, Brown Norway; Lung parenchymal strip

1. Introduction

We have recently described a marked and selective augmentation of the bronchoconstrictor response to adenosine following antigen challenge in actively sensitized Brown Norway (BN) rats (Fozard and Hannon, 2000; Hannon et al., 2001). Parenchymal strips prepared from lungs removed from actively sensitized BN rats challenged with allergen also show hyperresponsiveness to adenosine (Hannon et al., 2001). Both the bronchoconstrictor response to adenosine in vivo and the contractile response on the parenchymal strip ex vivo are mast cell mediated (Hannon et al., 2001, 2002; Fozard et al., 2003). On the basis of the ex vivo studies mentioned above, the potentiation of adenosine by allergen was postulated to reflect a local action on the mast cells of the lung.

The aim of the present work was to provide further evidence that the mast cells of the lung are the site of action for the interaction between allergen and adenosine. To this end, we have removed the lungs from actively sensitized animals and explored the interaction between allergen and adenosine on the parenchymal strip under entirely in vitro conditions. To further implicate the mast cell in the phenomenon, we investigated the effect of compound 48/80 (condensation product of *N*-methyl-*p*-methoxyphenylethylamine with formaldehyde), a non-immunological mast cell degranulating agent which directly activates the GTP binding protein, Gi, (Mousli et al., 1990) on the sensitivity of the strip to adenosine.

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2. Methods

2.1. Animals

Male BN rats weighing 200–300g were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland). They were kept at an ambient temperature of 22±2 C under a 12 h normal phase light–dark cycle and fed on NAFAG® pellets supplied by Nahr und Futtermittel, Gossau, Switzerland. Drinking water was freely available. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

2.2. Sensitization and challenge with allergen

The procedure is based on that described by Tarayre et al. (1992). Ovalbumin (20 μg ml^{-1}) was mixed (30 min on ice) in a blender (Polytron, Kinematica) with aluminium hydroxide (20 mg ml^{-1}) and injected (0.5 ml per animal s.c.). Injection of ovalbumin, together with adjuvant, was repeated 14 and 21 days later. Sensitized animals were used in experiments between days 28 and 35.

2.3. Lung parenchymal strip

Naïve rats or rats sensitized to ovalbumin, as described above, were killed by exposure to carbon dioxide. The lungs were perfused in situ via a cannula inserted into the pulmonary artery with 60 ml of modified Krebs' solution of the following composition—mM: NaCl, 118; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. The lungs were removed and four slices (10–12 mm long, 3 mm thick) were cut from the left lobe. Tissues were set up for recording isotonic tension in 10 ml organ baths containing modified Krebs' solution at 37 °C bubbled with 95% O_2/5% CO_2. Resting tension was maintained at 1 g.

2.3.1. Challenge with allergen or compound 48/80

After a stabilisation period of 1 h, during which time the tissues were repeatedly washed, a supramaximal concentration of bethanechol (0.1 mM) was added. After repeated washing during 1–1.5 h, a single concentration of OA (10 μg ml^{-1}) or compound 48/80 (1 mg ml^{-1}) or vehicle (saline) was added to the bath followed 30 min later by addition of adenosine (1 mM). In some experiments, a response to 5-hydroxytryptamine (5-HT; 100 nM), which gave a similar sized response to adenosine (1 mM), was generated in place of the adenosine. After washing the tissue repeatedly during 1 h, a concentration-response curve to 5-HT (100 nM–0.1 mM) was established followed a further 1 h later by a curve to bethanechol (100 nM–0.1 mM). Tension changes were expressed relative to the response to bethanechol, 0.1 mM.

2.3.2. Effects of methysergide and iralukast

Compounds or their vehicles were included in the bath fluid 30 min before the addition of ovalbumin or compound 48/80 and again 30 min before the start of the concentration response curves to 5-HT and bethanechol.

2.4. Materials

Aluminium hydroxide was from Merck, Germany and ovalbumin was from Fluka, Switzerland. Bethanechol (carbamyl-β-methyl-choline chloride), compound 48/80, 5-hydroxytryptamine creatinine sulfate and adenosine hemisulfate were obtained from Sigma, Switzerland. Methysergide maleate and iralukast were synthesised at Novartis, Basel, Switzerland. Adenosine and bethanechol were made up in water. All other drugs were dissolved in dimethylsulfoxide (DMSO). The final bath concentration of DMSO was 0.2%.

2.5. Data processing

All data are presented as means±S.E. means. Student's *t*-test for paired data was used to determine the significance of the difference between mean values. The Hochberg correction was applied to adjust for multiple comparisons. A P value <0.05 was considered significant.

3. Results

3.1. Effects of ovalbumin and adenosine on the lung parenchymal strip

Addition of ovalbumin (10 μg ml^{-1}) or saline to strips from non-sensitized animals did not induce any change in tension of the tissues (data not illustrated) and addition of adenosine (1 mM) 30 min following ovalbumin or saline induced quantitatively similar, small contractile responses (7.9±2.0%, n=3 and 8.2±1.0%, n=3 of the response to bethanechol, 0.1 mM, respectively). Incubation of strips from actively sensitized animals with saline induced no contractile effects (Fig. 1A and B). In contrast, strips removed from actively sensitized animals responded to ovalbumin (10 μg ml^{-1}) with a biphasic contractile response; a rapidly developing first phase lasting 2–5 min was followed by a slowly developing second phase lasting >30 min (Fig. 1A). Addition of adenosine (1 mM) 30 min following saline induced a small contractile response. Responses to adenosine (1 mM) were markedly increased during the second phase of the response to ovalbumin (Fig. 1A and B). In, contrast, the response to 5-HT (100 nM) which gave a monophasic response in saline-treated animals similar in magnitude to that seen with adenosine (1 mM; Fig. 1B) was slightly but significantly reduced following OA compared with saline pretreatment (Fig. 1C).

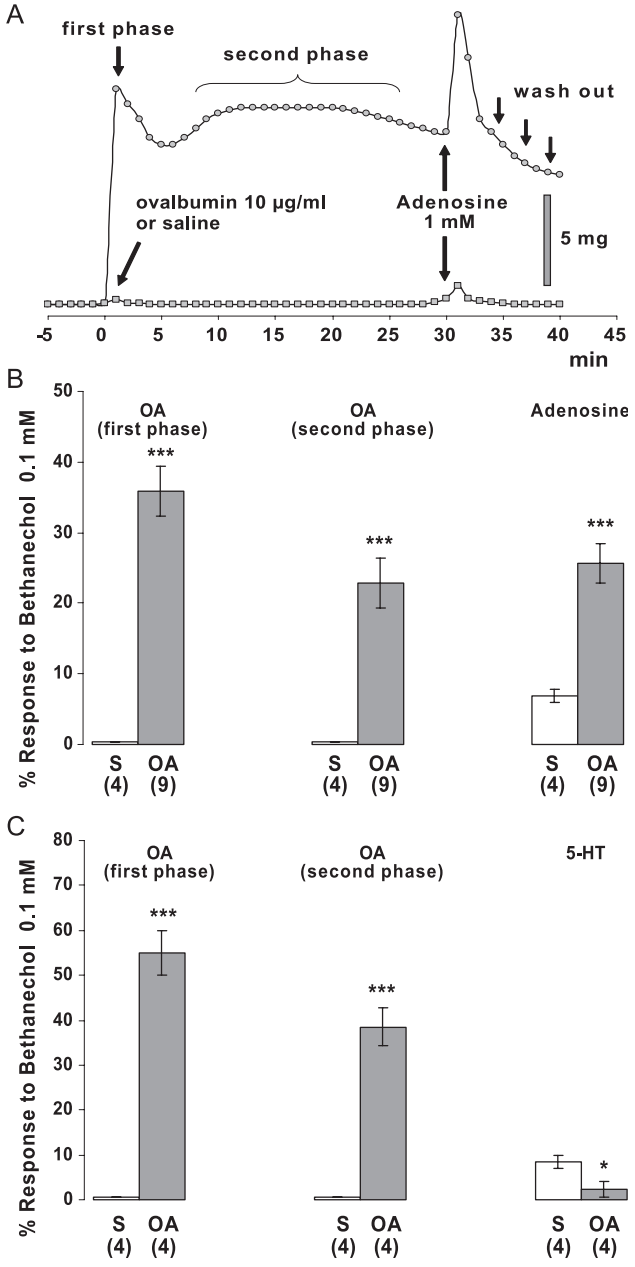

Fig. 1. Interaction between ovalbumin (OA) and adenosine or 5-HT on lung parenchymal strips prepared from actively sensitized Brown Norway rats. (A) Representative experimental records from tissues treated with ovalbumin (10 µg ml^{-1}) or saline. (B) Mean data showing responses to ovalbumin (10 µg ml^{-1}) and adenosine (1 mM) in tissues exposed to saline (S) or ovalbumin. (C) Similar experiment to (B) except that 5-HT (100 nM) was given instead of adenosine. Mean values (±S.E.M.) from the number of individual experiments shown in parentheses are presented. *P<0.05 and ***P<0.001 show that the value differs significantly from the equivalent value in the saline-treated group.

3.2. Effects of methysergide and iralukast on responses to ovalbumin and adenosine

The data are presented in Fig. 2. Incubation of strips from actively sensitized animals with methysergide (30 nM) markedly reduced the response to 5-HT (0.1 mM, the maximum concentration used in generating the cumulative concentration-response curve—Fig. 2D) and the first phase of the response to ovalbumin (Fig. 2A) but had no effect on the second phase of the response to ovalbumin (Fig. 2B). In contrast, the second phase of the response to ovalbumin was abolished by iralukast (1 µM) and unaffected by methysergide (30 nM; Fig. 2B). The augmented contractile response to adenosine was almost completely inhibited by methysergide and not significantly affected by iralukast (Fig. 2C).

3.3. Interaction between compound 48/80 and adenosine on the lung parenchymal strip

At concentrations of 10 and 100 µg ml^{-1}, compound 48/80 had no contractile effects on the parenchymal strip (data not illustrated). At 1 mg ml^{-1}, compound 48/80 induced contractions of the strip (11.5±1.4% of the response to bethanechol, 0.1 mM, n=8) which were short-lived (<10 min) and abolished in the presence of methysergide (30 nM, n=4; data not shown). Responses to adenosine (1 mM) were markedly and significantly (P<0.001) increased 30 min following compound 48/80 (1 mg ml^{-1}) compared to those in saline-treated strips (Fig. 3A). In contrast, the response to 5-HT (100 nM) was reduced, though not significantly, by pretreatment with compound 48/80. The enhanced response to adenosine was markedly and significantly (P<0.001) reduced in the presence of methysergide (30 nM; Fig. 3B).

4. Discussion

In previous studies, we showed a marked and selective enhancement of the bronchoconstrictor response to adenosine in actively sensitized BN rats following challenge with allergen. Similar augmented contractile responses to adenosine are also seen ex vivo on parenchymal strips removed from sensitized animals challenged 3 h before death with ovalbumin (Fozard and Hannon, 2000; Hannon et al., 2001). In each case, mast cells were implicated in the response and, on the basis of the ex vivo finding, the assumption was made that adenosine interacted with the mast cells present in the airways (Hannon et al., 2001, 2002). The present study in which the interaction between allergen and adenosine was investigated under entirely in vitro conditions provides new evidence in support of this assumption.

The response of the lung parenchymal strip removed from sensitized BN rats to ovalbumin has been described previously and the underlying mechanism of action investigated (Nagase et al., 1995). In contrast to the findings of these authors, which showed monophasic responses to ovalbumin, the response to the allergen in our study was clearly biphasic. The difference most likely represents the higher ovalbumin concentration used by Nagase et al. (1995) which was 100-fold greater than that used in our

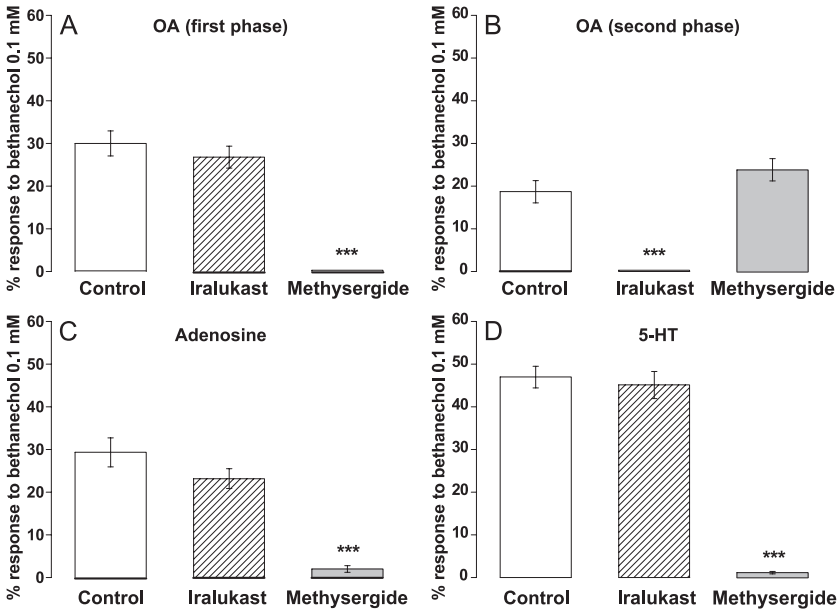

Fig. 2. Effects of methysergide (30 nM) or iralukast (1 μM) or vehicle (control) on responses of the lung parenchymal strip to ovalbumin, 10 μg ml^{-1} (A—first phase; B—second phase), adenosine, 1 mM (C) and 5-HT, 0.1 mM (D). For clarity, only the effect on the response to the maximum concentration of 5-HT (0.1 mM) used in establishing the cumulative concentration-response curve, is presented. Mean values (±S.E.M.) from eight (control), seven (iralukast) and three (methysergide) individual experiments are presented. ***P<0.001 shows that the value differs significantly from the equivalent value in the control group.

study. Nevertheless, Nagase et al. clearly showed the response to ovalbumin to be mediated by both 5-HT and leukotrienes and they concluded on this basis that mast cells

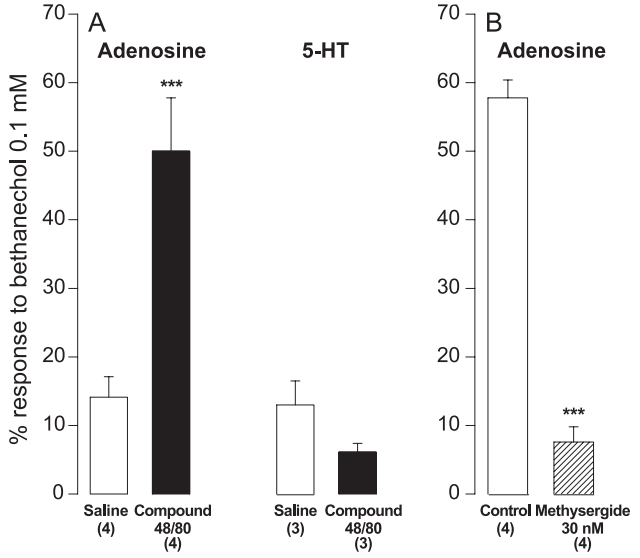

Fig. 3. Interaction between compound 48/80 and adenosine or 5-HT on lung parenchymal strips prepared from actively sensitized Brown Norway rats. (A) Data showing responses to adenosine (1 mM) or 5-HT (100 nM) in tissues exposed to saline or compound 48/80 (1 mg ml^{-1}). (B) Effect of methysergide, 30 nM, on the response to adenosine enhanced in the presence of compound 48/80. Mean values (±S.E.M.) from the number of individual experiments shown in parentheses are presented. ***P<0.001 shows that the value differs significantly from the equivalent value in the saline-treated/control group.

were indispensible for the response. Our data show that it is the early peak in response to ovalbumin which is 5-HT mediated and the more prolonged contractile phase which is mediated by cysteinyl leukotrienes. The release of 5-HT and leukotrienes from sensitized rat mast cells in response to allergen challenge has been reported (see, e.g. Macdonald et al., 1989; Hodson and Oliveira, 1996). The present results indicate that both phases of the contractile response of the parenchymal strip induced by ovalbumin are a consequence of activation of mast cells.

The response to adenosine was markedly increased following challenge with ovalbumin in vitro. Blockade of the enhanced response to adenosine by methysergide but not iralukast shows that it is mediated by 5-HT and not leukotrienes. Since the sensitivity of the strip to 5-HT is not increased, potentiation of adenosine cannot reflect an increase in the sensitivity of the bronchial smooth muscle to this mast cell mediator. This mirrors precisely the situation in the in vivo or ex vivo experiments where augmented bronchoconstrictor or parenchymal contractile responses to adenosine are mediated by 5-HT (Hannon et al., 2001).

Our present data generated under entirely in vitro conditions consolidate the case for the mast cell of the airways being the source of the 5-HT which mediates the response to adenosine augmented following allergen challenge. First, the lungs are perfused free of blood before being removed from the animals, which rules out an involvement of blood-borne sources of 5-HT such as platelets and basophils. Second, following ovalbumin challenge under entirely in vitro conditions, there can be no possibility of an influx of inflammatory cells into

the lung. Thus, inflammatory cells such as eosinophils associated with experimental allergic pulmonary inflammation in this model (Ellis et al., 2004) cannot be the source of the 5-HT released by adenosine. Third, as noted above, the cellular site of action of ovalbumin is the mast cell. The fact that the contractile response to the directly acting spasmogen, 5-HT, is not augmented following challenge with ovalbumin whereas that to adenosine is, implicates the mast cell in the augmented response to adenosine.

Our data with compound 48/80 provide further support for an involvement of the mast cell in the response to adenosine. Like allergen, compound 48/80 induces mast cell degranulation in the rat and the release of inflammatory mediators including 5-HT (Mazingue et al., 1978; Hannon et al., 1995; Fozard et al., 1996; Ferjan et al., 1997). In in vitro studies, compound 48/80 has been shown to degranulate mast cells in rat trachea and induce methysergide-sensitive contraction (Ikawati et al., 2000). The mechanism of mast cell degranulation induced by compound 48/80 is, however, non-immunological and involves direct activation of the GTP binding proteins of the Gi family (Mousli et al., 1990; Chahdi et al., 2000). Our data show that compound 48/80 induces contraction of the parenchymal strip which was abolished by methysergide indicating the involvement of mast cells. Moreover, as was the case with allergen, potentiation of adenosine, but not 5-HT, was evident 30 min after exposure to compound 48/80. The similarity between ovalbumin and compound 48/80 in enhancing responses to adenosine implies a common mechanism which is likely to be activation of the mast cell of the airways. Ample evidence from animal studies documents the synergy between adenosine receptor activation and allergen or compound 48/80 in inducing mast cell degranulation (Marquardt et al., 1978, 1994; Marquardt and Walker, 1990; Peachell et al., 1991; Ramkumar et al., 1993; Konnaris et al., 1996; Auchampach et al., 1997; Fozard and Hannon, 2000; Salvatore et al., 2000; Laffargue et al., 2002).

In our earlier studies, potentiation of adenosine by allergen was suggested to involve a discrete lung-based population of mast cells containing and releasing mainly 5-HT and brought into play by prior exposure to allergen (Hannon et al., 2001). The present findings showing potentiation of adenosine following both immunological and non-immunological mast cell activation under entirely in vitro conditions provides strong evidence in support of this conclusion.

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