ELSEVIER

European Journal of Pharmacology 406 (2000) 469–476

ejp

www.elsevier.nl/locate/ejphar

Effects of wortmannin on bronchoconstrictor responses to adenosine in actively sensitised Brown Norway rats

Bruno Tigani, Jason P. Hannon, Lazzaro Mazzoni, John R. Fozard *

Research Department, Novartis Pharma AG, Basel 4002, Switzerland

Received 29 June 2000; received in revised form 30 August 2000; accepted 7 September 2000

Abstract

The bronchoconstrictor response to adenosine in the actively sensitised Brown Norway rat is markedly augmented following low level allergen (ovalbumin) challenge. The response reflects activation of the A_{2B} receptor subtype and is mediated by 5-hydroxytryptamine (5-HT) released as a consequence of mast cell activation. We describe here the effects of wortmannin, a potent inhibitor of phosphatidylinositol-3-kinase and mast cell exocytosis, on the response to adenosine. Bronchoconstrictor responses to adenosine elicited 3 h following ovalbumin challenge were markedly and dose-dependently reduced by wortmannin given intratracheally (i.t.), 1 h prior to or 2 h post-allergen challenge. Responses to methacholine, which activates bronchial smooth muscle directly, and 5-HT were also reduced following wortmannin but to a lesser extent than those to adenosine. Bronchoconstrictor responses to adenosine 3 h post-challenge with vehicle were also markedly reduced by wortmannin given intratracheally (i.t.), 1 h prior to the "sham" challenge. Plasma histamine and 5-HT levels increased in response to adenosine given 3 h following ovalbumin challenge. The increases were suppressed by wortmannin given i.t., 2 h post-ovalbumin challenge. A reduction in the sensitivity of the airways to 5-HT explains in part the reduced bronchoconstrictor response to adenosine induced by wortmannin. A direct action to suppress 5-HT release from airway mast cells induced by adenosine also contributes to the reduction in the response. Inhibition of phosphatidylinositol-3-kinase is the presumed mechanistic basis for the observed effects.

Keywords: Phosphatidylinositol-3-kinase; Wortmannin; Adenosine; Methacholine; 5-HT; Bronchoconstriction; Active sensitisation; Ovalbumin; Brown Norway, rat

1. Introduction

We have recently described a marked and selective augmentation of the bronchoconstrictor response to adenosine following allergen challenge in actively sensitised, Brown Norway rats (Hannon et al., 1999a,b; Fozard and Hannon, 2000). Pharmacological analysis indicated that the response is mediated by the adenosine A_{2B} receptor subtype (Hannon et al., 1999b) and is primarily a consequence of mast cell activation (Hannon et al., 1999a).

Phosphatidylinositol-3-kinase is activated in mast cells following cross-linking of the high affinity receptor for immunoglobulin E (IgE), FcεRI (Yano et al., 1993; Marquardt et al., 1996; Hirasawa et al., 1997; Pendl et al., 1997; Bhattacharyya et al., 1998), exposure to carbachol or thapsigargin (Hirasawa et al., 1997) and is critically involved in the signal transduction pathway responsible for degranulation and the release of bioactive mediators. A key finding implicating phosphatidylinositol-3-kinase in an obligatory role in mast cell degranulation induced by IgE-dependent and independent mechanisms is that secretory responses can be abolished by low concentrations of wortmannin, a potent inhibitor of phosphatidylinositol-3-kinase (Ui et al., 1995; Marquardt et al., 1996; Hirasawa et al., 1997; Cardenas et al., 1998; Cissel et al., 1998).

We sought to provide further insight into the mechanism of the potentiation of adenosine by allergen challenge, by using wortmannin as a tool to prevent the activation of mast cells. Our data show wortmannin to be an effective blocker of the mast cell-mediated bronchoconstrictor response to adenosine. The mechanism appears to be independent of the activation of mast cells by allergen.

A part of the results was presented to a meeting of the British Pharmacological Society in July, 1999 (Tigani et al., 1999).

* Corresponding author. Tel.: +41-61-324-6772; fax: +41-61-324-2733.

E-mail address: john_r.fozard@pharma.novartis.com (J.R. Fozard).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00705-6

2. Methods

2.1. Animals

Male Brown Norway rats weighing 200–300 g were used throughout this study. Groups of up to six animals were housed in sawdust lined drawer cages (approx. 560 × 335 × 200 mm) and kept at an ambient temperature of 22 ± 2°C under 12 h normal phase light–dark cycles. They were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) 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).

2.1.1. Sensitisation procedure

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.) coincidentally with *Acullulare pertussis* adsorbat vaccine (0.2 ml per animal i.p.; diluted 1:4 with saline 0.9%). Injection of ovalbumin, together with adjuvant, was repeated 14 and 21 days later. Sensitised animals were used in experiments between days 28 and 35.

2.2. Measurement of lung function

Animals were anaesthetised with sodium pentothal (70 mg kg^{-1} i.p.) and a tracheotomy performed. Heparinised polyethylene catheters were inserted into the left carotid artery for recording mean arterial blood pressure and into the left jugular vein for drug administration. To suppress spontaneous respiration, animals were given an intramuscular injection of vecuronium bromide (12 mg kg^{-1}). No experiment lasted longer than 90 min, during which time surgical anaesthesia was maintained without the need for supplementary anaesthesia. Body temperature was maintained at 37°C with a heated pad, controlled by a rectal thermistor.

Animals were ventilated (7 ml kg^{-1}, 1 Hz) via the tracheal cannula with a mixture of air and oxygen (50:50, v/v). Ventilation was monitored at the trachea by a pneumotachograph (Fleisch 0000, Zabona, Switzerland) in line with the respiratory pump and connected to a differential pressure transducer (MP 4514871, Validyne, USA). Coincident pressure changes within the thorax were measured via an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne, USA). The difference between the inflation and pleural pressures was taken as a measure of transpulmonary pressure. From measurements of airflow and transpulmonary pressure, airway resistance (R_L, cm H_2O l^{-1} s^{-1}) was calculated after each respiratory cycle by use of a digital electronic pulmonary monitoring system (PMS, Mumed, London, UK). Mean arterial blood pressure (and heart rate by derivation) was recorded from the carotid artery by means of a pressure transducer (P23Dd, Gould, USA).

2.3. Definition of bronchoconstrictor sensitivity

In a first series of experiments, bronchoconstrictor responses to adenosine (0.3 and 1 mg kg^{-1} i.v., or 3 and 10 mg kg^{-1} i.v. depending on the experiment — see Results) and methacholine (3 and 10 μg kg^{-1} i.v.) were established sequentially in groups of actively sensitised Brown Norway rats challenged intratracheally (i.t.) 3 h previously with vehicle (saline, 0.2 ml) or ovalbumin (0.3 mg kg^{-1}). The interval between the two adenosine doses was 15 min. Fifteen minutes after the second adenosine dose, methacholine was administered with a 2-min interval between doses. In a second series of experiments, bronchoconstrictor responses to adenosine (0.3 and 1 mg kg^{-1} i.v. or 3 and 10 mg kg^{-1} i.v. depending on the experiment — see Results) and 5-hydroxytryptamine (5-HT; 3, 10 and 30 μg kg^{-1} i.v.) were established in groups of actively sensitised animals challenged i.t. either with vehicle (saline, 0.2 ml) or ovalbumin (0.3 mg kg^{-1} i.t.). There was a 15-min interval between the two doses of adenosine. Fifteen minutes after the second dose of adenosine, 5-HT was administered with a 2-min interval between doses.

2.4. Measurement of histamine and 5-HT in plasma

Animals were anaesthetised with sodium pentothal (70 mg kg^{-1} i.p.), and polyethylene catheters placed in both the left carotid artery (for blood collection) and right jugular vein (for drug administration). After set-up, the animals were left for a stabilisation period of at least 20 min. The details of the experimental intervention are given in the Results section. Blood samples (approximately 1 ml) were taken into 1.5 ml potassium ethylenediamine tetraacetate (EDTA)-coated plastic collection tubes and chilled on ice. Samples were immediately centrifuged (1700 × *g*, 30 min, 4°C; Omnifuge 2.0, Heraeus Sepratech, CH) and the overlying plasma aspirated and stored at −30°C prior to assay.

The concentrations of histamine and 5-HT in the plasma were assessed by colorimetric assay using commercial kits (Immunotech). The assays are based on acetylation of the biogenic amine and competition between this acylated product and acetylcholinesterase-coupled amine for a monoclonal antibody coated onto microwells of a 96-well microtitre plate. Bound acetylcholinesterase activity is estimated by degradation of a chromogenic substrate and the absorbency was read immediately at 405 nm (Labsystem Multiskan Plus, Bioconcept, CH). The concentrations of both histamine and 5-HT in the samples were calculated as nanomolar, according to their activity with respect to the

standard curve and presented as nanograms per milliliter of plasma.

2.5. Materials

Aluminium hydroxide was obtained from Merck, Germany and *A. pertussis* adsorbat vaccine was from the Vaccinal and Serotherapic Institute in Bern, Switzerland. Pentothal (thiopentalum natricum) was obtained from Abbott, Switzerland. Norcuron (vecuronium bromide) was from Organon Teknika, Holland. Ovalbumin was obtained from Fluka, Switzerland. Wortmannin ([1*S*-(1α,6*b*α,9*a*β, 11α,11*b*β)]-11(acetyloxy)-1,6*b*,7,8,9*a*,10,11,11*b*-octahydro1(methoxymethyl)-9*a*,11*b*-dimethyl-3*H*-furo[4,3,2-de]indeno[4,5-*h*]-2-benzopyran-3,6,9-trione), methacholine chloride and adenosine hemisulphate were obtained from Sigma, Switzerland. Wortmannin (10 mg) was dissolved in 100% dimethlysulphoxide and diluted with 0.9% w/v NaCl to 100 μg ml^{-1}. All other compounds were prepared in 0.9% w/v NaCl.

2.6. Data analysis

All data are presented as means ± S.E.M. Statistical analysis was performed by means of Student's *t* test for unpaired data. *P* values < 0.05 were considered significant.

3. Results

3.1. Effect of wortmannin on bronchoconstrictor responses to adenosine and methacholine in actively sensitised Brown Norway rats challenged with ovalbumin

The design of the first experiment was based on our observation that bronchoconstrictor responses to adenosine in actively sensitised Brown Norway rats are markedly enhanced 3 h following challenge with a small dose of ovalbumin (0.3 mg kg^{-1} i.t.) and are indirect, a consequence of mast cell activation. By contrast, responses to methacholine, which are the result of direct, muscarine-receptor-mediated activation of the bronchial smooth muscle, are not altered by ovalbumin challenge (Hannon et al., 1999a,b; Fozard and Hannon, 2000).

Bronchoconstrictor responses to adenosine, 0.3 and 1 mg kg^{-1}, elicited 3 h following ovalbumin challenge, were unchanged by pretreatment with wortmannin, 1 and 3 μg kg^{-1}, but were markedly and dose-dependently reduced by wortmannin 10 and 100 μg kg^{-1} given i.t., 1 h prior to allergen challenge (i.e. 4 h before challenge with adeno-

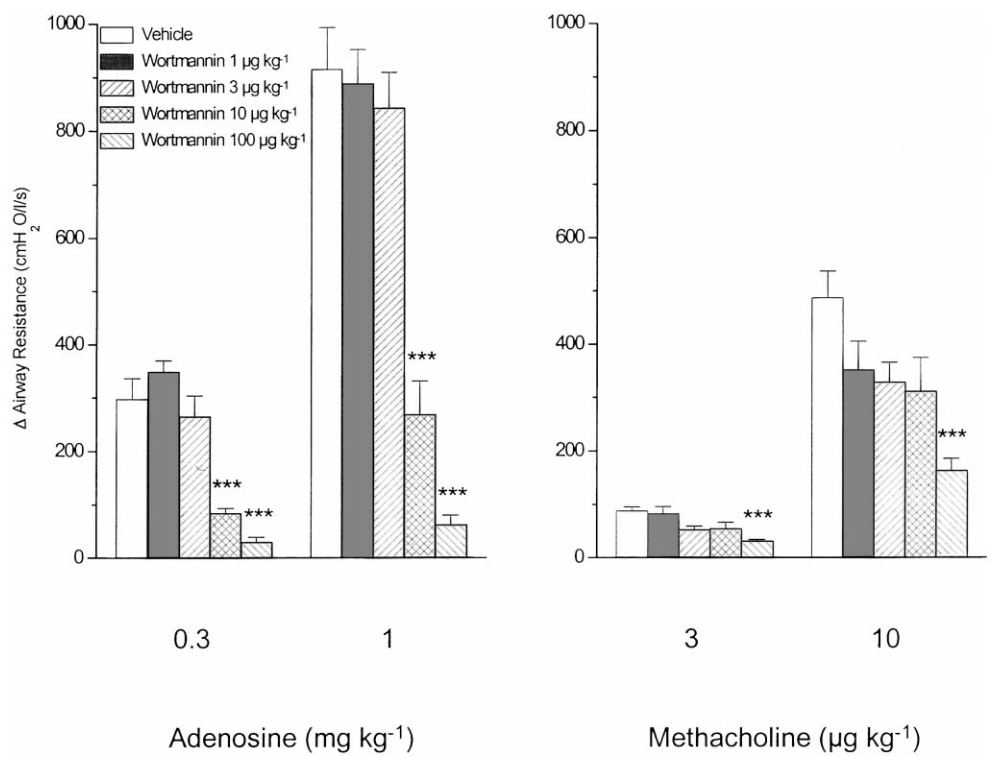

Fig. 1. Bronchoconstrictor responses to adenosine and methacholine in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent the incremental increases in airway resistance induced by adenosine (0.3 and 1 mg kg^{-1} i.v.) and methacholine (3 and 10 μg kg^{-1} i.v.) measured 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin i.t., at the doses indicated, 1 h prior to ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. There were no significant differences in mean baseline airway resistance values between vehicle- and wortmannin-treated animals.

sine). Responses to methacholine, 3 and 10 μg kg^{-1}, were minimally affected following wortmannin, 1, 3, or 10 μg kg^{-1} but were reduced significantly by approximately 70% following the 100 μg kg^{-1} dose (Fig. 1). The falls in blood pressure and heart rate induced by adenosine and methacholine were unaffected by any of the doses of wortmannin (data not illustrated).

In a second experiment, wortmannin (10 μg kg^{-1}) was administered 2 h following challenge with ovalbumin, 0.3 mg kg^{-1} i.t., and responses to adenosine and methacholine elicited 1 h later. The results were qualitatively and quantitatively similar to those obtained when the same dose of wortmannin was given 1 h prior to challenge with ovalbumin. Thus, bronchoconstrictor responses to adenosine were strongly inhibited whereas those to methacholine were reduced to a markedly lesser extent (Fig. 2).

Actively sensitised Brown Norway rats challenged with vehicle in place of ovalbumin will respond to adenosine with mast cell-dependent bronchoconstriction although higher doses (3–10 mg kg^{-1} i.v.) are required (Hannon et al., 1999a). Responses to adenosine, 3 and 10 mg kg^{-1}, elicited 3 h following vehicle (saline, 0.2 ml) challenge, were markedly reduced by wortmannin 10 μg kg^{-1} given i.t., 1 h prior to the 'sham' challenge. In this paradigm, responses to the higher dose of methacholine (10 μg kg^{-1} i.v.) were also significantly reduced following wortmannin but again to a markedly lesser extent than the mast cell-dependent responses to adenosine (Fig. 3).

3.2. Effect of wortmannin on bronchoconstrictor responses to adenosine and 5-HT in actively sensitised Brown Norway rats challenged with ovalbumin

In actively sensitised rats challenged with ovalbumin, the bronchoconstrictor response to adenosine is mediated by 5-HT released from airway mast cells (Hannon et al., 1999a). Thus, to further evaluate the selectivity of action of wortmannin, experiments were carried out with 5-HT as the comparative bronchospasmogen. The results are shown in Fig. 4. In confirmation of the results of the earlier experiments (Figs. 1 and 2), responses to adenosine were strongly inhibited following intratracheal administration of wortmannin, 10 μg kg^{-1}, given either 1 h before (Fig. 4) or 2 h after (Fig. 5) ovalbumin challenge. However, in contrast to its effects on methacholine, which were minimal, wortmannin inhibited the responses to 5-HT significantly and appreciably, although again, not to the same extent as adenosine (Figs. 4 and 5).

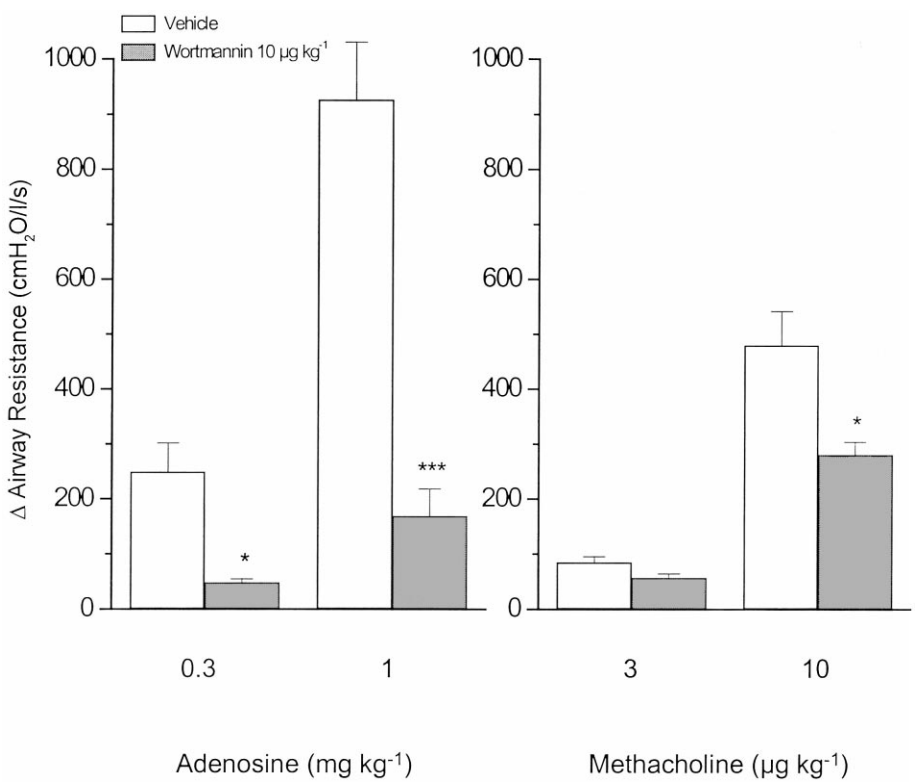

Fig. 2. Bronchoconstrictor responses to adenosine and methacholine in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent the incremental increases in airway resistance induced by adenosine (0.3 and 1 mg kg^{-1} i.v.) and methacholine (3 and 10 μg kg^{-1} i.v.) measured 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 μg kg^{-1} i.t.) 2 h post-ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$, $^{***}P < 0.001$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. There were no significant differences in mean baseline airway resistance values between vehicle- and wortmannin-treated animals.

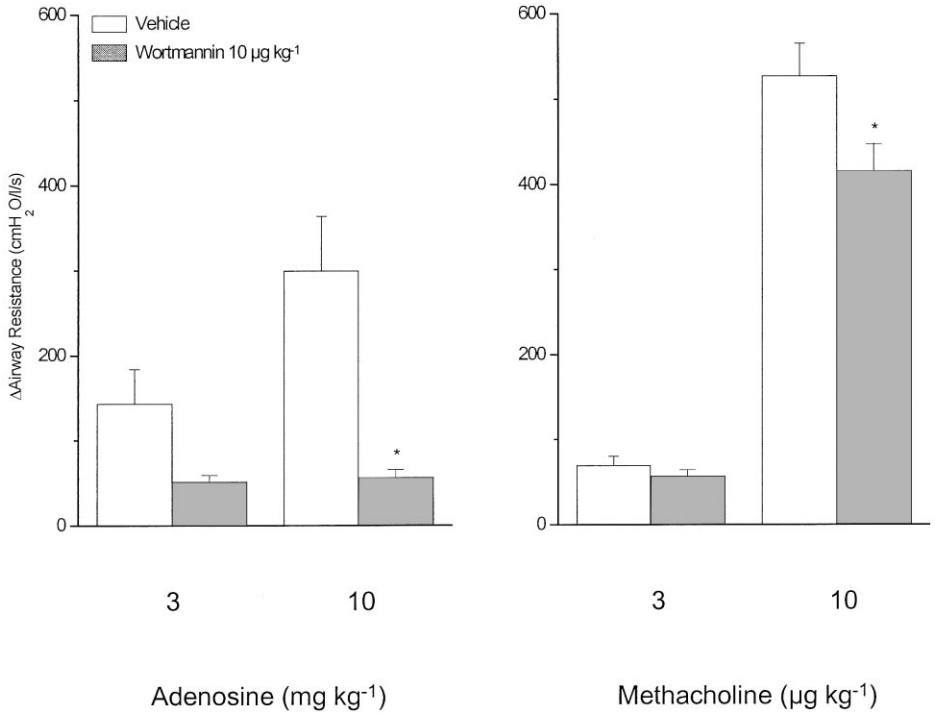

Fig. 3. Bronchoconstrictor responses to adenosine and methacholine in actively sensitised vehicle (saline, 0.2 ml i.t.; "sham" challenge)-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent the incremental increases in airway resistance induced by adenosine (3 and 10 mg kg^{-1} i.v.) and methacholine (3 and 10 µg kg^{-1} i.v.) measured 3 h after challenge of sensitised Brown Norway rats with vehicle for ovalbumin. Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 µg kg^{-1} i.t.) 1 h prior to the "sham" challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. There were no significant differences in mean baseline airway resistance values between vehicle- and wortmannin-treated animals.

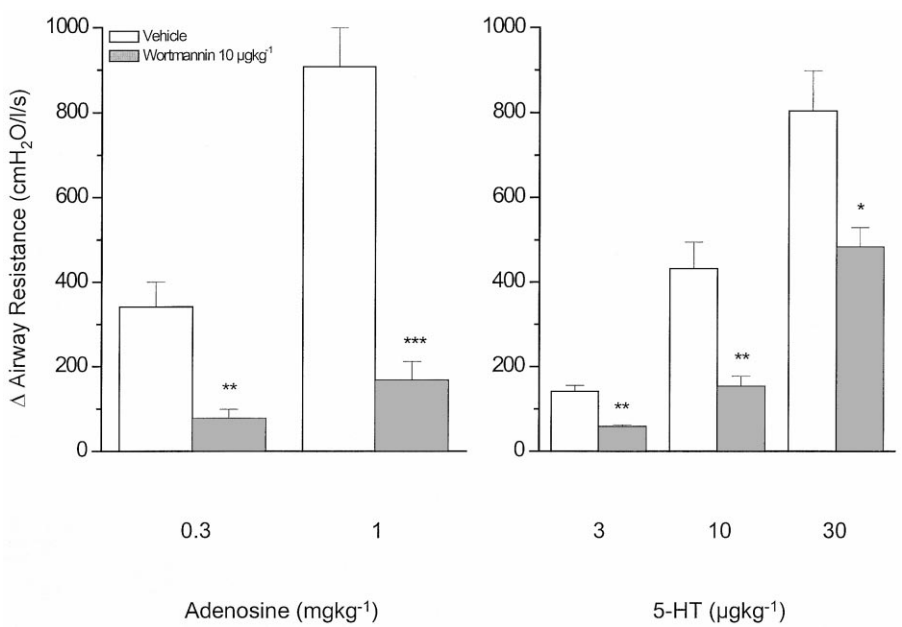

Fig. 4. Bronchoconstrictor responses to adenosine and 5-HT in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent the incremental increases in airway resistance induced by adenosine (0.3 and 1 mg kg^{-1} i.v.) and 5-HT (3, 10 and 30 µg kg^{-1} i.v.) measured 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 µg kg^{-1} i.t.) 1 h prior to ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. There were no significant differences in mean baseline airway resistance values between vehicle- and wortmannin-treated animals.

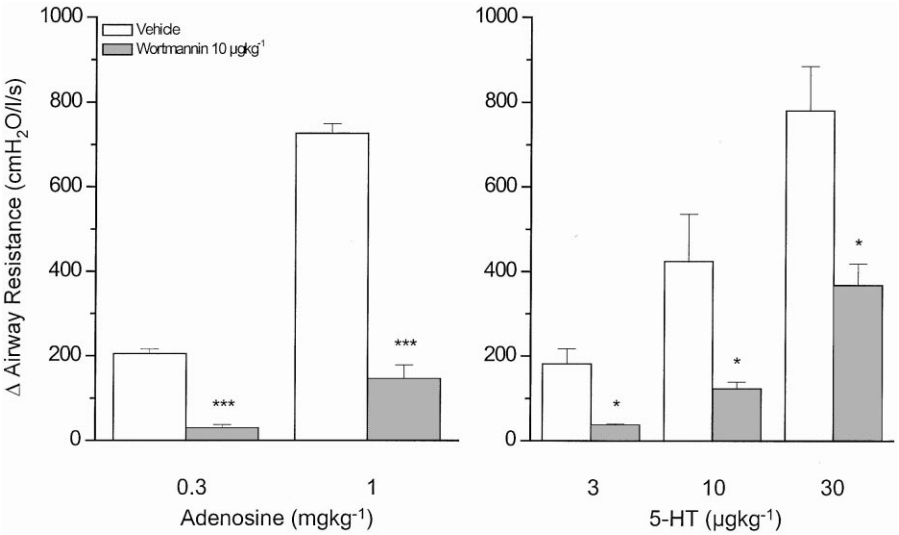

Fig. 5. Bronchoconstrictor responses to adenosine and 5-HT in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent the incremental increases in airway resistance induced by adenosine (0.3 and 1 mg kg^{-1} i.v.) and 5-HT (3, 10 and 30 μg kg^{-1} i.v.) measured 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 μg kg^{-1} i.t.) 2 h post-ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group. There were no significant differences in mean baseline airway resistance values between vehicle- and wortmannin-treated animals.

3.3. Effects of wortmannin on the increases in plasma histamine and 5-HT concentrations induced by adenosine in actively sensitised Brown Norway rats challenged with ovalbumin

In actively sensitised Brown Norway rats challenged with ovalbumin (0.3 mg kg^{-1} i.t.) 3 h prior to testing, adenosine (1 mg kg^{-1} i.v.) induces marked increases in the plasma concentrations of both histamine and 5-HT; the response is suppressed by disodium cromoglycate implicating the mast cell as the source of these mediators (Hannon et al., 1999a). The effect of wortmannin on the release of histamine and 5-HT induced by adenosine was therefore measured according to the following paradigm. Actively sensitised animals were challenged with ovalbumin (0.3 mg kg^{-1} i.t.) and 2 h later given wortmannin (10 μg kg^{-1}), or vehicle (saline, 0.2 ml) by the i.t. route. A further 1 h later, a blood sample was taken. Adenosine (1

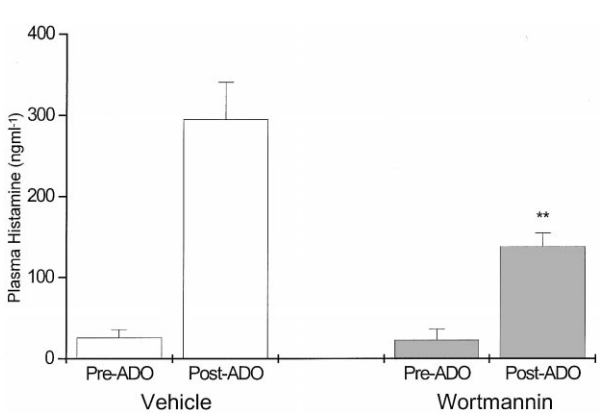

Fig. 6. Plasma histamine levels in response to adenosine in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent plasma concentration of histamine induced by adenosine (ADO, 1 mg kg^{-1} i.v.) administered 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 μg kg^{-1} i.t.) 2 h post-ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{**}P < 0.01$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group.

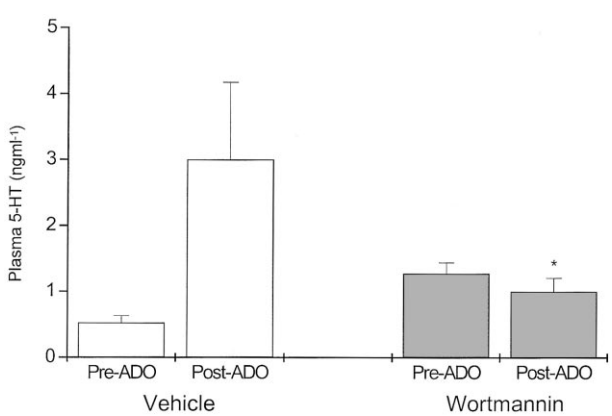

Fig. 7. Plasma 5-HT levels in response to adenosine in actively sensitised ovalbumin-challenged Brown Norway rats: Effect of pretreatment with wortmannin. Columns represent plasma concentrations of 5-HT induced by adenosine (ADO, 1 mg kg^{-1} i.v.) administered 3 h after challenge of sensitised Brown Norway rats with ovalbumin (0.3 mg kg^{-1} i.t.). Groups of animals were given either vehicle (saline, 0.2 ml) or wortmannin (10 μg kg^{-1} i.t.) 2 h post-ovalbumin challenge. The mean (±S.E.M.) of four individual values is presented. $^{*}P < 0.05$ indicates that the value is significantly different from the equivalent value in the vehicle-treated group.

mg kg^{-1}) or vehicle (saline, 0.2 ml) was injected i.v. 5 min later. A second blood sample was taken 1 min later and assayed for plasma histamine and 5-HT concentrations. Wortmannin inhibited significantly the increase in histamine induced by adenosine and essentially abolished the rise in 5-HT (Figs. 6 and 7).

4. Discussion

We have previously shown that the bronchoconstrictor response to adenosine in the actively sensitised Brown Norway rat is markedly increased 3 h after challenge with a small dose of allergen which does not itself induce bronchoconstriction (Hannon et al., 1999a; Fozard and Hannon, 2000). The augmented response is a consequence of adenosine A_{2B} receptor activation and is mast cell-mediated (Hannon et al., 1999a,b). One possible explanation for the up-regulation of mast cell responsiveness to adenosine is that the small dose of allergen primes or partially activates the mast cell such that a second stimulus to degranulation is rendered more effective. In order to test the hypothesis, we administered wortmannin, a selective inhibitor of phosphatidylinositol-3-kinase, which blocks mast cell degranulation in response to cross-linking of the high affinity receptor for IgE, FcεRI, to sensitised rats 1 h prior to challenge with allergen. Consistent with the hypothesis, the bronchoconstrictor response to adenosine was blocked by a low dose of wortmannin and blockade was selective in that responses to methacholine (which reflect a direct action on the bronchial smooth muscle) were only minimally affected.

The conclusion that wortmannin is acting to inhibit the response to adenosine exclusively by preventing mast cell activation by the preceding challenge with allergen is, however, called into question by our subsequent analysis. First, wortmannin proved able to inhibit selectively the response to adenosine when administered 2 h after allergen challenge. Clearly, 2 h after allergen challenge, suppression of the initial activation of the mast cells by allergen would not be possible. Indeed, it has been clearly demonstrated in in vitro studies that wortmannin cannot inhibit mast cell degranulation already in progress (Marquardt et al., 1996). Second, wortmannin shows potent blocking activity against the response to high doses of adenosine in actively sensitised animals challenged with vehicle rather than allergen. Such responses are also mast cell-mediated (unpublished observations) and the data thus identify an effect of wortmannin on the response to adenosine which is independent of the influence of allergen.

One possible explanation could be that activation of the mast cell occurred normally after wortmannin but that the responsiveness of the airways to 5-HT, the mediator of the response to adenosine (Hannon et al., 1999a), was suppressed. In support of this, significant inhibition of bronchoconstrictor responses to 5-HT was indeed seen following wortmannin. Although this was somewhat less than that seen with adenosine, there can be little doubt that inhibition of the airways sensitivity to 5-HT contributes substantially to the inhibitory effect of wortmannin on adenosine-induced bronchoconstriction. We have no explanation for why responses to 5-HT are suppressed to a greater extent than those to methacholine. In in vitro studies, 5-HT has been shown to contract rat airways directly by activating $5\text{-}HT_2$ receptors on airway smooth muscle and to a minor extent indirectly, by activation of $5\text{-}HT_2$ receptors on parasympathetic nerve endings to cause the release of acetylcholine (Szarek et al., 1995). However, under the conditions of our experiments, bronchoconstrictor responses to 5-HT were unaffected by atropine suggesting an effect directly on the airway smooth muscle (unpublished observations).

On the other hand, measurement of the plasma concentrations of histamine and 5-HT establishes that wortmannin, given 2 h following allergen challenge in actively sensitised Brown Norway rats, does markedly reduce (histamine) or abolish (5-HT) the increase in mast cell mediators induced by adenosine which underlies its bronchoconstrictor effect (Hannon et al., 1999a). As argued above, this effect cannot be attributed to suppression of mast cell activation induced by allergen and a direct effect to inhibit adenosine receptor activation of the airway mast cells has to be countenanced. There are precedents in the literature for IgE-independent mast cell degranulation responses, which are wortmannin-sensitive. Thus, degranulation of mouse bone marrow-derived mast cells induced by the non-selective adenosine receptor agonist, *N*-ethylcarboxaminoadenosine, or the calcium ionophore, A23187, was blocked by low concentrations of wortmannin (Marquardt et al., 1996). Similarly in rat basophilic RBL-2H3 cells, wortmannin proved a powerful inhibitor of degranulation induced by carbachol or thapsigargin (Hirasawa et al., 1997; Cissel et al., 1998). Our data show, for the first time, that an IgE-independent stimulus leading to degranulation of mast cells can be suppressed by wortmannin in vivo.

Can the inhibitory effect of wortmannin in these studies be attributed to blockade of phosphatidylinositol-3-kinase? While an unequivocal answer cannot be given, the answer is likely to be yes based on the potency and selectivity of wortmannin. Thus, the dose of wortmannin used to inhibit the response to adenosine is low ($ED_{50} < 10$ μg kg^{-1} i.t.). This, plus the fact that wortmannin shows > 50-fold selectivity for phosphatidylinositol-3-kinase over other mammalian kinases, such as myosin-light chain kinase (Cardenas et al., 1998), strongly supports the above interpretation.

In the Brown Norway rat, mast cell-dependent bronchoconstrictor responses to adenosine are mediated by adenosine A_{2B} receptors (Hannon et al., 1999b). Mast cells from mouse (Marquardt et al., 1994), dog (Auchampach et al., 1997), and man (Feoktistov and Biaggioni,

1995) bear adenosine A_{2B} receptors, which couple via G_q to phospholipase C. Activation of this pathway leads to increases in diacylglycerol and inositol trisphosphate (IP_3), activation of protein kinase C and mobilisation of calcium leading to degranulation and mediator release (Feoktistov and Biaggioni, 1997). Activation of protein kinase C and an increase in intracellular calcium are necessary and sufficient signals for secretion (Beaven and Ozawa, 1996) and the question arises as to where in this sequence is the wortmannin-sensitive step. The most likely mechanism is inhibition of the calcium-induced activation of phospholipase D, which appears to be the primary enzyme for the generation of diglycerides for activation of protein kinase C and secretion (Cissel et al., 1998). The calcium-induced activation of phospholipase D is suppressed by wortmannin at nanomolar concentrations, which are considered selective for phosphatidylinositol-3-kinase (Cissel et al., 1998).

In conclusion, wortmannin is a potent inhibitor of both the bronchoconstrictor response to adenosine augmented following allergen challenge and the increase in histamine and 5-HT concentrations in plasma induced by adenosine in actively sensitised Brown Norway rats. A reduction in the sensitivity of the airways to 5-HT explains in part the reduced bronchoconstrictor response to adenosine. A direct action to suppress mediator release from airway mast cells induced by adenosine also contributes to the reduction in the response. Inhibition of phosphatidylinositol-3-kinase is the presumed mechanistic basis for the observed effects.

References

Auchampach, J.A., Jin, X., Wan, T.C., Caughey, G.H., Linden, J., 1997. Canine mast cell adenosine receptors: cloning and expression of the A_3 receptor and evidence that degranulation is mediated by the A_{2B} receptor. Mol. Pharmacol. 52, 846–860.

Beaven, M.A., Ozawa, K., 1996. Role of calcium, protein kinase C and MAP kinase in the activation of mast cells. Allergol. Int. 45, 73–84.

Bhattacharyya, S.B., Drucker, I., Reshef, T., Kirshenbaum, A.S., Metcalfe, D.D., Mekori, Y.A., 1998. Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact. J. Leukocyte Biol. 63, 337–341.

Cardenas, M.E., Sanfridson, A., Cutler, N.S., Heitman, J., 1998. Signal transduction cascades as targets for therapeutic intervention by natural products. Trends Biotechnol. 16, 427–433.

Cissel, D.S., Fraundorfer, P.F., Beaven, M.A., 1998. Thapsigargin-induced secretion is dependent on activation of a cholera toxin-sensitive and PI3-kinase-regulated phospholipase D in a mast cell line. J. Pharmacol. Exp. Ther. 285, 120–128.

Feoktistov, I., Biaggioni, I., 1995. Adenosine A_{2B} receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. J. Clin. Invest. 96, 1979–1986.

Feoktistov, I., Biaggioni, I., 1997. Adenosine A_{2B} receptors. Pharmacol. Rev. 49, 381–402, (In press).

Fozard, J.R., Hannon, J.P., 2000. Species differences in adenosine induced bronchoconstrictor responses. Clin. Exp. Allergy 30, 1213–1220.

Hannon, J.P., Tigani, B., Mazzoni, L., Fozard, J.R., 1999a. Mechanism of airway hyperreactivity to adenosine induced by allergen challenge in actively sensitised Brown Norway rats. Br. J. Pharmacol. 127, 76P.

Hannon, J.P., Tigani, B., Mazzoni, L., Fozard, J.R., 1999b. The receptor mediating airway hyperreactivity to adenosine induced by allergen challenge in actively sensitised Brown Norway rats. Br. J. Pharmacol. 127, 77P.

Hirasawa, N., Sato, Y., Yomogida, S., Mue, S., Ohuchi, K., 1997. Role of phosphatidylinositol 3-kinase in degranulation induced by IgE-dependent and -independent mechanisms in rat basophilic RBL-2H3 cells. Cell. Signalling 9, 305–310.

Marquardt, D.L., Walker, L.L., Heinemann, S., 1994. Cloning of two adenosine receptor subtypes from mouse bone marrow derived mast cells. J. Immunol. 152, 4508–4515.

Marquardt, D.L., Alongi, J.L., Walker, L.L., 1996. The phosphatidylinositol 3-kinase inhibitor wortmannin blocks mast cell exocytosis but not IL-6 production. J. Immunol. 156, 1942–1945.

Pendl, G.G., Eva, E.P., Werner, T., Harrer, N.E., Auer, M., Baumruker, T., 1997. Effects of phosphatidylinositol 3-kinase inhibitors on degranulation and gene induction in allergically triggered mouse mast cells. Int. Arch. Allergy Immunol. 112, 392–399.

Szarek, J.L., Zhang, J.Z., Gruetter, C.A., 1995. Mechanisms of 5-hydroxytryptamine-induced contraction of isolated rat intrapulmonary bronchi. Pulm. Pharmacol. 8, 273–281.

Tigani, B., Hannon, J.P., Mazzoni, L., Fozard, J.R., 1999. Effect of wortmannin on antigen-induced airway inflammation and bronchoconstrictor responsiveness in actively sensitised Brown Norway rats. Br. J. Pharmacol. 128, 111P.

Ui, M., Okada, T., Hazeki, K., Hazeki, O., 1995. Wortmannin as a unique probe for an intracellular signalling protein, phosphoinositide 3-kinase. Trends Biotechnol. 20, 303–307.

Yano, H., Nakanishi, S., Kimura, K., Hanai, N., Saitoh, Y., Fukui, Y., Nanomura, Y., Matsuda, Y., 1993. Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells. J. Biol. Chem. 268, 25846–25856.