



Allergic lung inflammation induces pulmonary vascular hyperresponsiveness

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ABSTRACT: Pulmonary arterial vasoconstriction is an important early component of pulmonary hypertension. Inflammatory mechanisms play a prominent role in the pathogenesis of pulmonary hypertension. The present authors investigated the potential role of acute allergic lung inflammation for alterations in pulmonary haemodynamics.

BALB/c mice were intraperitoneally sensitised to ovalbumin and challenged by ovalbumin inhalation. Subsequently, lungs were ventilated and perfused *ex vivo*, and pulmonary arterial pressure (P_{pa}) was continuously monitored.

Isolated perfused lungs of allergen-sensitised and -challenged mice showed five-fold enhanced P_{pa} responses to serotonin, which is reported to be a significant contributor to pulmonary hypertension in humans. This increase in P_{pa} was abolished by the serotonin receptor-2A antagonist ketanserin, but not the serotonin receptor-1B antagonist GR127935. Intracellular signalling to serotonin involved phosphatidylcholine-specific phospholipase C and protein kinase C, as well as Rho-kinase, as assessed by employing the specific inhibitors D609, bisindolylmaleimide and Y27632, respectively. In addition to serotonin, impressively enhanced P_{pa} increases in allergic lungs were also evoked by the thromboxane receptor agonist U46619, angiotensin II and endothelin-1.

In conclusion, allergic lung inflammation was accompanied by impressive pulmonary vascular hyperresponsiveness. These results suggest a possible role for allergic inflammation in the development of pulmonary arterial hypertension.

KEYWORDS: Allergic inflammation, 5-hydroxytryptamine, isolated mouse lung, ovalbumin, pulmonary hypertension, serotonin

Inflammatory airway disorders, inflammation is not restricted to the airways, but may also affect pulmonary vessels. Examination of lungs from patients who died during an asthma attack revealed that inflammation, including eosinophilia, involves the tissue and large pulmonary arteries adjacent to bronchi [1]. Moreover, in lungs from long-term smokers, morphological changes in pulmonary arteries in parallel with small airway disease and emphysema were found [2], and inflammatory mechanisms are assumed to contribute to alterations of the pulmonary circulation in chronic obstructive pulmonary disease (COPD) [3, 4]. Against this background, it is noteworthy that a variety of autoimmune disorders and infectious diseases have been reported to be associated with pulmonary arterial hypertension (PAH) [5]. Taken together, inflammatory mechanisms may, therefore, play an important role in the pathogenesis of PAH [5].

Structural and functional changes of the pulmonary circulation in PAH include remodeling of the

pulmonary arterial wall, endothelial dysfunction and thrombosis [6], as well as pulmonary vasoconstriction, which is assumed to be an important early component of the hypertensive process [7]. In this context, the vasoconstrictive agent serotonin (5-hydroxytryptamine; 5-HT) has been identified as a relevant contributor to PAH [7]. Medication with the appetite suppressants aminorex fumarate and fenfluramine, resulting in elevated plasma 5-HT, was associated with an increased incidence of PAH [8, 9], but the predisposing factors that are essential for the manifestation of the disease are unknown [10].

In order to model pulmonary hypertension in rodents, intravascular monocrotaline application has been widely used, leading to neutrophilic pulmonary vascular inflammation, pulmonary hypertension and cor pulmonale [11]. Perivascular immune responses with leukocyte infiltration were also observed in a variety of airway inflammation models [12]. In particular, in a mouse model of allergic airway inflammation, extensive

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eosinophil and mononuclear cell infiltrates were seen around the pulmonary blood vessels [13, 14], and a recent study in mice showed that central features of allergen-induced airway remodeling were also present in the pulmonary vessels, including smooth muscle enlargement [15]. These findings provide further evidence that, beyond airway alterations, allergic inflammation may also affect the pulmonary vascular bed. However, the functional consequences of these findings remain unknown.

In the current study, it was observed that allergen-induced lung inflammation induced increased pulmonary arterial pressor responses to 5-HT measured in isolated perfused mouse lungs. The present authors investigated the contribution of the 5-HT_{2A} and 5-HT_{1B/D} receptor, addressed the 5-HT specificity of the observed phenomenon, and analysed the intracellular signalling pathways related to the 5-HT induced pulmonary vascular responses.

METHODS

Allergen sensitisation and challenge

All experimental procedures were approved by local authorities. Pathogen-free female BALB/c mice (20–23 g; BgVV, Berlin, Germany) were maintained on an ovalbumin (OVA)-free diet. Systemic sensitisation with OVA (20 µg·injection⁻¹; Sigma, Deisenhofen, Germany) adsorbed to 2 mg Al(OH)₃ (Pierce, Rockford, IL, USA) by *i.p.* injections on days 0 and 14, and repeated airway challenges with aerosolised 1% OVA (weight/volume) in PBS (20 min) on days 28, 29 and 30 were performed as described [16] (fig. 1).

Preparation of bronchoalveolar lavage fluid

Animals were killed 24 h after the last airway challenge, and the tracheas were cannulated. Airways underwent lavage twice with 1 mL of ice-cold PBS, and cell numbers were determined as previously described [17]. Total leukocytes, lymphocytes, eosinophils and macrophages were differentiated in bronchoalveolar lavage fluid (BALF) according to morphological criteria [17].

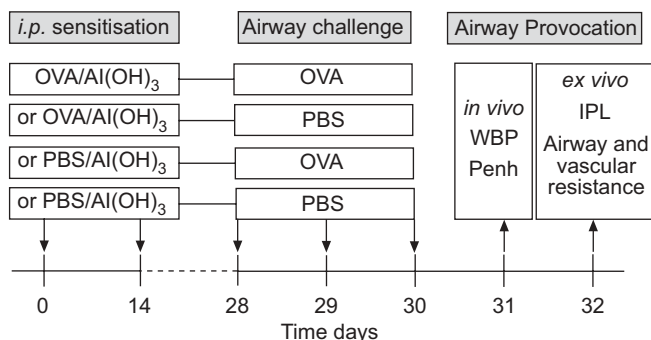


FIGURE 1. Diagram to show the animal treatment protocol. Mice were sensitised to ovalbumin (OVA) adsorbed to Al(OH)₃, by *i.p.* injections or received vehicle on days 0 and 14. On indicated days, airway challenges were performed with 1% OVA or solvent (PBS), delivered by aerosolisation for 20 min. The airway response to methacholine was measured *in vivo* on day 31 by means of whole body plethysmography (WBP) determining “enhanced pause” (Penh). On day 32, *ex vivo* airway and vascular responses were determined in the isolated perfused and ventilated mouse lung (IPL).

Histochemistry

Lung tissue was fixed in 4% formalin, dehydrated, mounted in paraffin, sectioned and stained with periodic acid-Schiff (PAS) reaction using a standard protocol.

Serum levels of total and OVA-specific immunoglobulin E

On day 31, serum levels of total and OVA-specific immunoglobulin (Ig)E were measured by means of ELISA, as previously described [18]. Levels of OVA-specific IgE were related to pooled standards generated in the authors’ laboratory and expressed as arbitrary units per mL. Total serum IgE (Sigma, Taufkirchen, Germany) levels were calculated by comparison with known mouse IgE standards and expressed in ng·mL⁻¹.

In vivo airway responsiveness

Airway responsiveness (AR) was measured in unrestrained animals by barometric whole body plethysmography (Buxco®; EMKA Technologies, Paris, France) as described elsewhere [16]. As an index of *in vivo* airway obstruction, enhanced pause (Penh) values were calculated [16].

Isolated perfused mouse lung

Mouse lungs were prepared as described previously [19, 20]. Lungs were perfused with 37°C sterile Krebs-Henseleit-hydroxyethylamyllopectine buffer (1 mL·min⁻¹; Serag-Wiesner, Naila, Germany) in a nonrecirculating fashion, and left atrial pressure was adjusted at +2.2 cmH₂O. Pulmonary arterial pressure (*P*_{pa}) and venous pressure were continuously monitored and digitised. Following isolation, lungs were ventilated by constant negative pressure (expiration: 4.5 cmH₂O to inspiration -9.0 cmH₂O; 90 breaths·min⁻¹) in a closed chamber. Hyperinflation (-24 cmH₂O) was performed at 4-min intervals. The chamber pressure was continuously measured by a differential pressure transducer, and airflow velocity was monitored by means of a pneumotachograph connected to a second differential pressure transducer. Signals were amplified and registered with Pulmodyn® software, and the data for airway resistance (*R*_{aw}) were analysed as described previously [21]. All hardware and software were purchased from HSE Harvard Apparatus, March-Hugstetten, Germany.

Airway and vascular responsiveness in isolated perfused mouse lung

After a steady state period of 30 min, 5-HT (Sigma, Taufkirchen, Germany) or, alternatively, the thromboxane receptor agonist U46619, angiotensin II or endothelin-1 (all Calbiochem, Darmstadt, Germany) were administered to the perfusate for 30 s, 3, 2 or 10 min, respectively. The concentration of either agent was increased in 12-min intervals. *R*_{aw} and *P*_{pa} were determined 30 s before and at the respective maximum response after 5-HT, U46619, angiotensin II or endothelin-1 administration. The change in *R*_{aw} was expressed as fold *R*_{aw}, and the difference in *P*_{pa} (ΔP _{pa}) was expressed in cmH₂O.

Ketanserin (1 µM; Sigma, Taufkirchen, Germany), GR127935 (0.1 µM; donated by GlaxoSmithKline, Munich, Germany), D609 (100 µM; Sigma), xestospongine C (1 µM; Biomol, Hamburg, Germany), bisindolylmaleimide (10 µM), or Y27632 (5 µM; both Calbiochem, Darmstadt, Germany) were administered to the perfusate 10 min prior to 5-HT application.

Data analysis

Data are expressed as mean \pm SEM. Differences were analysed by ANOVA, followed by *post hoc* Student–Newman–Keuls test.

RESULTS**Allergen-induced lung inflammation**

On day 31, allergen-induced lung inflammation was evaluated. The total number of cells, as well as the number of eosinophils and lymphocytes, was increased in BALF of OVA/OVA mice compared with all other groups (fig. 2a). Plasma levels of total IgE and OVA-specific IgE were increased in OVA-sensitised animals compared with PBS/PBS and PBS/OVA animals (fig. 2b). OVA/OVA mice showed increased *in vivo* AR, whereas OVA/PBS, PBS/OVA and PBS/PBS treated mice displayed low AR, as assessed by measuring Penh in unrestrained animals by barometric whole body plethysmography after inhalative challenges with increasing methacholine doses

(data not given). PAS stained lung sections of OVA/OVA mice revealed peribronchial and perivascular infiltrates (fig. 2c).

Airway and vascular responsiveness to 5-HT in isolated perfused mouse lung

On day 32 of the protocol, lungs were ventilated and perfused *ex vivo*. 5-HT infusion induced a large, rapid and dose-dependent P_{pa} increase in the lungs of OVA-sensitised and OVA-challenged mice, whereas lungs of all control groups displayed only minor P_{pa} changes (fig. 3a). Lungs of OVA/OVA mice responded immediately to 5-HT perfusion, with P_{pa} peaking after 1 min and reaching baseline values within <4 min ($\leq 10^{-5}$ M 5-HT) or within 10–20 min (10^{-4} M 5-HT). Importantly, in the isolated lungs, neither baseline P_{pa} values (OVA/OVA 8.83 ± 0.23 cmH₂O; OVA/PBS 9.28 ± 0.31 cmH₂O; PBS/OVA 7.98 ± 0.50 cmH₂O; PBS/PBS 8.79 ± 0.37 cmH₂O), nor baseline values for airway resistance (OVA/OVA 1.50 ± 0.06

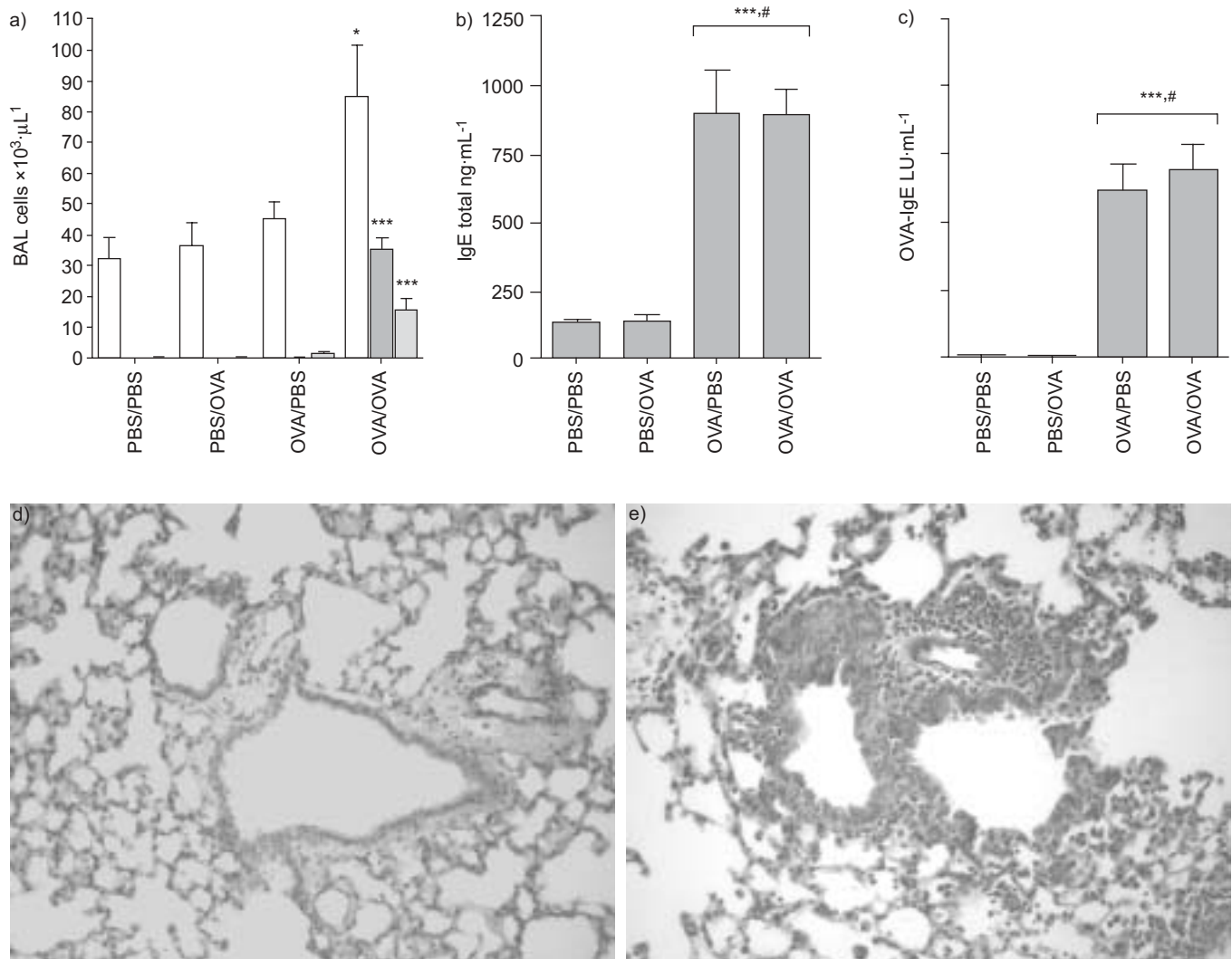


FIGURE 2. Allergic lung inflammation following ovalbumin (OVA)-sensitisation and challenge. Animals were sensitised and challenged as described in figure 1. a) Bronchoalveolar lavage (BAL) was performed for cell count ($n=8-10$). b) Day 31, blood was sampled for total immunoglobulin (IgE) detection ($n=4-8$) and c) ovalbumin (OVA)-specific IgE detection. d, e) Representative view of lung sections stained with haematoxylin-eosin and periodic acid-Schiff stain in PBS/PBS and OVA/OVA (d and e, respectively). \square : total cells; \blacksquare : eosinophils; \blacksquare : lymphocytes. Values are given as mean \pm SEM. Error bars are missing when falling into symbols. *: $p<0.05$; ***: $p<0.001$ versus all other groups. #: $p<0.001$ versus PBS/OVA.

cmH₂O·s⁻¹·mL⁻¹; OVA/PBS 1.52±0.08 cmH₂O·s·mL⁻¹; PBS/OVA 1.68±0.06 cmH₂O·s·mL⁻¹; PBS/PBS 1.55±0.08 cmH₂O·s·mL⁻¹) differed between the experimental groups. Isolated lungs of allergen-sensitised and -challenged mice showed enhanced AR to 5-HT as compared with all other experimental groups (fig. 3b).

5-HT induced vasoconstriction is mediated via the 5-HT_{2A} receptor

The current authors then analysed whether 5-HT contributed to the development of acute pulmonary vasoconstriction *via* the Gi-coupled 5-HT₁ receptor or *via* the Gq-coupled 5-HT_{2A} receptor. The specific 5-HT_{2A} receptor antagonist ketanserin reduced the 5-HT-induced *P*_{pa} responses by >95%. In contrast, the 5-HT₁-specific receptor antagonist GR127935 [22] did not affect the pressor response to 5-HT, neither in lungs from OVA-sensitised and -challenged mice nor in lungs from nonsensitised, nonchallenged controls (fig. 4).

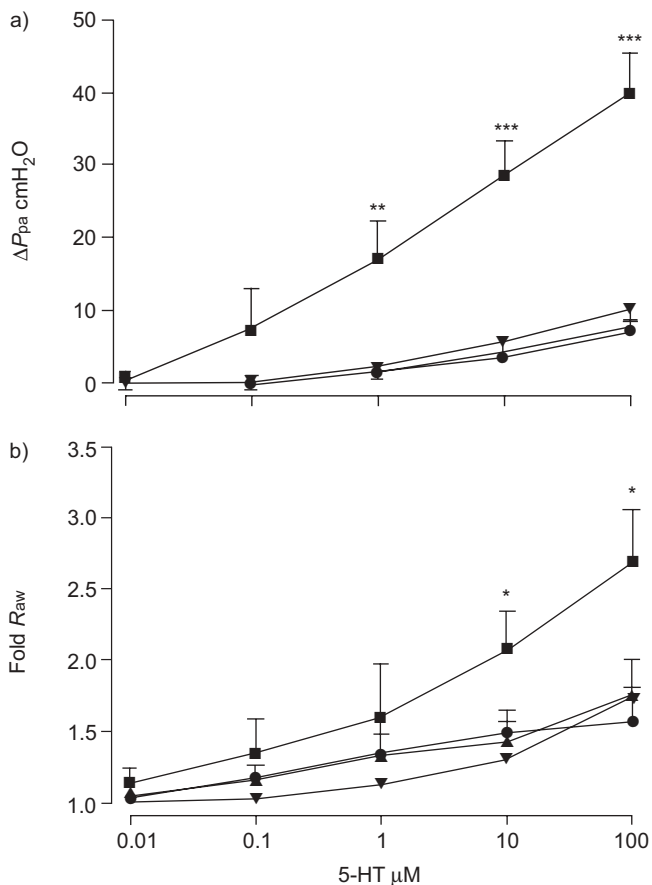


FIGURE 3. Increase of pulmonary arterial pressure (*P*_{pa}) and airway resistance (*R*_{aw}) in response to 5-hydroxytryptamine (5-HT) in isolated mouse lungs. Mice were sensitised and challenged as described in figure 1. Perfusion of the isolated lung was repetitively changed to buffer 5-HT (30 s each dose). *P*_{pa} (a) and *R*_{aw} (b) were determined considering values 30 s before 5-HT perfusion and at maximum *P*_{pa} and *R*_{aw}, respectively. ■: ovalbumin (OVA)/OVA; ●: PBS/PBS; ▲: OVA/PBS; ▼: PBS/OVA. Values are given as mean±SEM (n=6 each). Error bars are missing when falling into symbols. *: p<0.05; **: p<0.01; ***: p<0.001 *versus* all other groups.

Vascular hyperresponsiveness in isolated perfused mouse lungs is not 5-HT specific

Similarly to 5-HT, the vasoconstrictors thromboxane A₂, angiotensin II and endothelin-1 act *via* Gq-coupled receptors. When lungs were perfused with the thromboxane receptor agonist U46619 (3×10⁻⁹ to 3×10⁻⁸ M), *P*_{pa} in OVA/OVA lungs increased manifold as compared with all other groups (fig. 5a). Interestingly, AR to U46619 in OVA/OVA lungs was not different from all other groups (fig. 5b). Furthermore, in response to angiotensin II and endothelin-1, *P*_{pa} in OVA/OVA lungs showed higher increases compared with PBS/PBS lungs (fig. 5c and d).

Pressor response to 5-HT in OVA/OVA lungs mediated via phosphatidylcholine-specific phospholipase C and protein kinase C

Finally, the intracellular cascade involved in the signalling downstream of 5-HT was investigated. Pre-treatment of perfused OVA/OVA lungs with the phosphatidylcholine-specific phospholipase C (PC-PLC) inhibitor D609 largely attenuated the pressor responses to 5-HT (fig. 6). As D609 additionally inhibits sphingomyelin synthase [23], the current authors excluded a major importance of sphingomyelin synthesis for the pressor response by using the sphingomyelinase inactivator imipramine [24]. Imipramine did not significantly reduce the *P*_{pa} increase to 5-HT (5-HT: 46.5±10.83 cmH₂O; 5-HT+imipramine: 36.81±11.57 cmH₂O; p=0.69), suggesting that the pressor response depended on PC-PLC rather than sphingomyelin. Inhibition of a downstream effector of PC-PLC, inositol-1,4,5-triphosphate (IP₃),

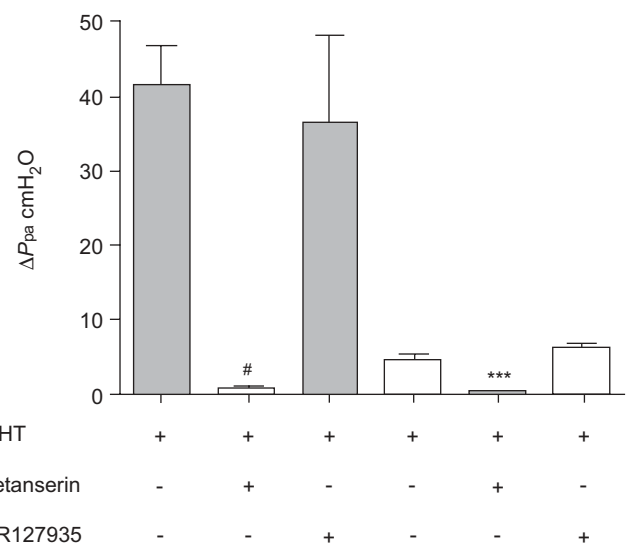


FIGURE 4. Increase of pulmonary arterial pressure (*P*_{pa}) in response to 5-hydroxytryptamine (5-HT) perfusion in presence or absence of the 5-HT_{1B} receptor antagonist GR127935 (0.1 μM), or the 5-HT_{2A} receptor antagonist ketanserin (1 μM). Mice were sensitised and challenged according to the protocol depicted in figure 1. Inhibitors were infused starting 10 min prior to perfusion with 5-HT (100 μM). Difference in *P*_{pa} (Δ*P*_{pa}) was determined considering values 30 s before 5-HT perfusion and at maximum *P*_{pa}. □: PBS/PBS; ■: ovalbumin (OVA)/OVA. Data are presented as mean±SEM (n=4 each). #: p<0.001 *versus* 5-HT- and 5-HT/GR127935-exposed OVA/OVA lungs; ***: p<0.001 *versus* 5-HT- and 5-HT/GR127935-exposed PBS/PBS lungs.

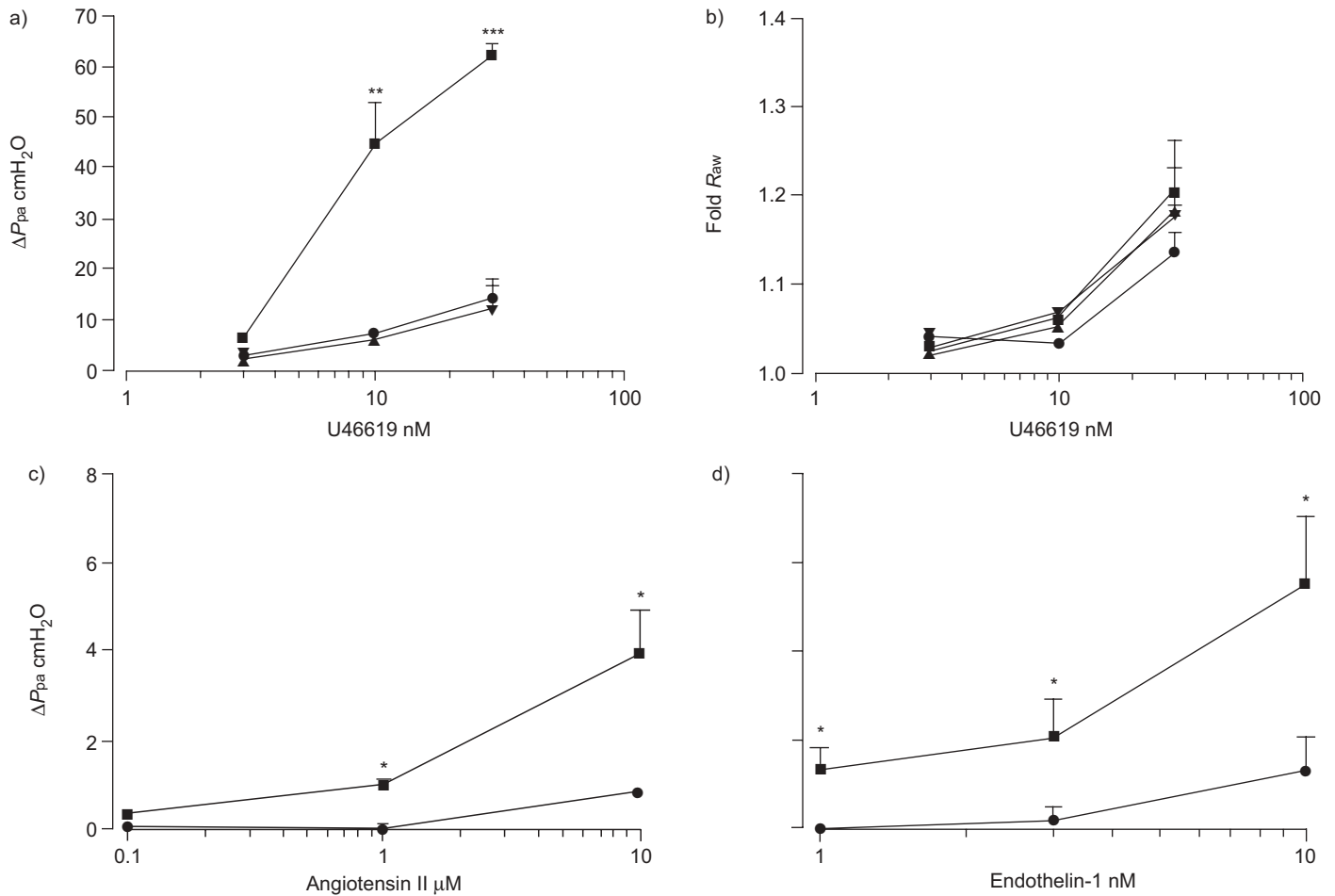


FIGURE 5. Increase of pulmonary arterial pressure (P_{pa}) in isolated mouse lungs in response to a) U46619 c) angiotensin II, d) endothelin-1, and b) airway resistance (R_{aw}) in response to U46619. Mice were sensitised and challenged as described in figure 1. Perfusion of the isolated lung was repetitively changed to buffer containing U46619 (3 min each dose), angiotensin II (2 min) or endothelin-1 (10 min). Difference in P_{pa} (ΔP_{pa} ; a, c, d) and R_{aw} (b) was determined considering values 30 s before perfusion with any agent and at maximum P_{pa} and R_{aw} , respectively. ■: ovalbumin (OVA)/OVA; ▲: OVA/PBS; ▼: PBS/OVA; ●: PBS/PBS. Data are presented as mean \pm SEM ($n=4-5$ each). Error bars are missing when falling into symbols. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ versus all other groups.

using the IP₃ receptor antagonist xestospongin C did not affect the 5-HT induced vasoconstriction. In contrast, pre-treatment with the selective protein kinase C (PKC) inhibitor bisindolylmaleimide almost completely abolished 5-HT-induced vasoconstriction in lungs from sensitised and challenged mice. Furthermore, blockade of the Rho-kinase pathway by the highly selective Rho-kinase inhibitor Y-27632 resulted in a distinct reduction of the P_{pa} increase (fig. 6).

DISCUSSION

The main findings of the current study were as follows. 1) Allergic lung inflammation induced by allergen-sensitisation and -challenge caused impressive pulmonary vascular hyperresponsiveness to 5-HT in mice. 2) The increased vascular response to 5-HT was mediated by the G_q-coupled 5-HT_{2A} receptor. 3) Allergen-induced pulmonary vascular hyperresponsiveness was not specific to 5-HT. 4) The intracellular signalling process in response to 5-HT involved PC-PLC and PKC, as well as Rho-kinase.

At present, the relevance of these findings for human disease remains unknown and it is not clear if allergen-induced

pulmonary vascular hyperresponsiveness parallels airway hyperresponsiveness (a cardinal feature of asthma [25]) in humans.

However, pulmonary arterial vasoconstriction is regarded as an important early step in pulmonary hypertension (PH) pathogenesis [7]. PH can be associated with diverse inflammatory diseases, including infections (HIV [26], human herpes virus 8 [27], schistosomiasis [28]) and autoimmune disorders (e.g. lupus erythematosus [29], scleroderma [30]), as well as COPD, suggesting a role for inflammatory mechanisms in PH [7]. In COPD, airway inflammation is assumed to affect the entire lung including the pulmonary vasculature [4], which might be relevant for PH pathogenesis. Moreover, in exacerbations of COPD triggered by respiratory infections, deterioration of cor pulmonale frequently occurs [3, 31].

There is only very little information concerning a link between allergic lung inflammation and pulmonary vascular responses, and the current study did not identify the exact mechanism. Due to the close vicinity of the pulmonary vessels and the airways, it is tempting to speculate that overspill of

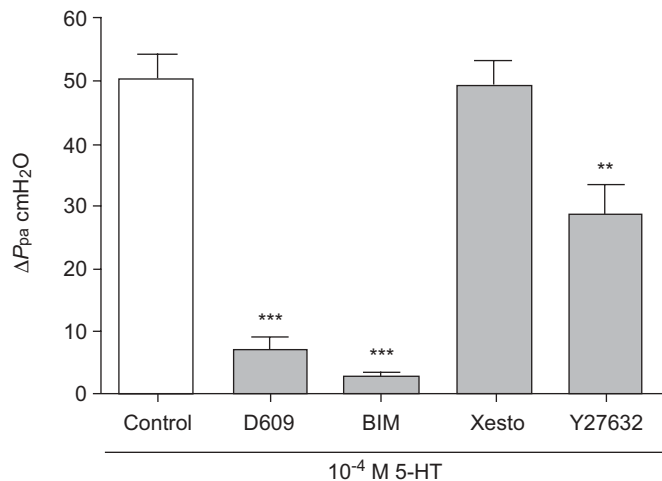


FIGURE 6. Increase of pulmonary arterial pressure (P_{pa}) in response to 5-hydroxytryptamine (5-HT) in presence (■) or absence (□) of specific inhibitors for phosphatidylcholine-specific phospholipase C (D609; 10 μ M), protein kinase C (bisindolylmaleimide (BIM); 10 μ M), the inositol triphosphate receptor antagonist xestospongine C (xesto; 1 μ M), or the Rho-kinase (Y27632; 5 μ M). Mice were sensitised and challenged according to the protocol depicted in figure 1. Inhibitors were infused starting 10 min prior to perfusion with 5-HT (100 μ M). Difference in P_{pa} (ΔP_{pa}) was determined considering values 30 s before 5-HT perfusion and at maximum P_{pa} . Data are presented as mean \pm SEM ($n=4$ each). **: $p<0.01$ versus control; ***: $p<0.001$ versus control.

inflammatory mediators [32] may contribute to vascular hyperresponsiveness. Notably, a recent study demonstrated that the alterations in the protein expression profile after allergen sensitisation and challenge partly correlated with those observed in hypoxia [33], which is known to induce PAH and increased vascular responses to vasoconstrictors [34, 35].

In a variety of airway inflammation models, perivascular immune responses were observed [12], and important features of airway remodeling were noted in the pulmonary vessels of allergen-sensitised and -challenged mice [15]. In order to investigate the impact of acute allergic lung inflammation on the pulmonary circulation, the current authors employed a well established murine model of acute allergen-sensitisation and challenge, which is characterised by several important features of allergic airway inflammation, including AR, eosinophil infiltration of the airways, and production of allergen-specific IgE [16, 36]. Histology showed peribronchial and perivascular leukocyte infiltrations, thus suggesting acute inflammation in the pulmonary vascular bed. Chronic allergen exposure, however, may have different biological impact on the pulmonary circulation, but was beyond the scope of the current study.

5-HT appears to be involved in the pathogenesis of PAH [7]. In the current model, 5-HT caused an immediate, dramatic P_{pa} increase in lungs of allergen-sensitised and -challenged mice in addition to enhanced bronchoconstriction. In control lungs, 5-HT evoked only a moderate vascular pressor response, which is in line with previous studies [21]. Acute vasoconstriction to 5-HT was mediated by the 5-HT_{1B} receptor in human pulmonary arteries *ex vivo* [37] and *in vivo* [38], whereas in normal rat pulmonary arteries, these responses were mediated

predominantly by the 5-HT_{2A} receptor [39]. In *ex vivo* perfused lungs of allergen-sensitised and -challenged mice, as well as control mice, the 5-HT-evoked P_{pa} responses were blocked by the 5-HT_{2A} receptor antagonist ketanserin, whereas 5-HT_{1B} receptor antagonism by GR127935 did not diminish vasoconstriction. Moreover, the 5-HT_{1B/D} receptor agonist sumatriptan (1–100 μ M) did not affect P_{pa} (data not given in detail). These results suggest an exclusive role of the 5-HT_{2A} receptor, without the modulatory function of the 5-HT_{1B} receptor described in chronic hypoxic rats [39] and mice [34], where 5-HT_{2A} (Gq-coupled) and 5-HT_{1B} (Gi-coupled) receptors were assumed to act synergistically [40].

In addition to 5-HT, thromboxane, endothelin-1 and angiotensin II, which also bind to G-protein coupled receptors in the pulmonary vasculature [40], have all been reported to cause acute pulmonary arterial vasoconstriction [21, 41, 42]. In the present study, low doses of the thromboxane receptor agonist U46619 caused an impressive P_{pa} response in lungs of allergen-sensitised and -challenged mice. As the employed doses were lower than reported to cause bronchial constriction in previous studies [21], airway resistance was only marginally affected by U46619. Therefore, a possible contribution of mechanical interaction between airway pressure and P_{pa} to the P_{pa} increase was excluded.

Furthermore, perfusion with endothelin-1 or angiotensin II also resulted in a pronounced arterial pressor response in OVA/OVA, as compared with PBS/PBS-lungs. Although these vasoconstrictors may not exclusively act *via* Gq-coupled receptors, the current results suggest an important role of this pathway.

Gq-coupled receptors activate PLC, thereby increasing cytosolic IP₃ [43] and diacylglycerol (DAG). IP₃ induces cytosolic Ca²⁺ increase, followed by myosin light chain (MLC) phosphorylation and subsequent smooth muscle contraction [43]. DAG activates PKC, leading to increased MLC phosphorylation *via* diverse mechanisms [43, 44]. By using specific inhibitors, the current authors demonstrated that the enhanced P_{pa} response to 5-HT in allergen-sensitised and -challenged murine lungs was PC-PLC and PKC dependent. In contrast, the IP₃-receptor antagonist xestospongine C did not influence the 5-HT-induced pressor response, although the employed concentration was three-fold above 50% of the median inhibitory concentration [45]. However, at this concentration xestospongine prevented acetylcholine-induced calcium-signalling in lung slices [46] and attenuated platelet activating factor-induced oedema formation in isolated lungs [47].

Beyond IP₃ and PKC, activated Rho-kinase indirectly increases MLC phosphorylation contributing to additional contractile force of myofilaments without changes in Ca²⁺ concentration [48]. Pre-treatment with the highly selective Rho-kinase inhibitor Y27632 [49] reduced the enhanced pressor response significantly. Interestingly, augmented contribution of Rho-kinase to airway smooth muscle contraction in allergen-sensitised guinea pigs has recently been described [50]. Moreover, Rho-kinase was upregulated in carotid arteries of spontaneously hypertensive rats [51] and in coronary arteries of interleukin-1 β -treated pigs [52], leading to vascular hyperresponsiveness to 5-HT. Against this background, a role for the

Ca²⁺ sensitising Rho-kinase pathway in allergen-induced pulmonary vascular hyperresponsiveness may be assumed.

Concerning the role of 5-HT in human airways, several issues remain unsettled. Interestingly, in addition to being implicated in PH pathogenesis, serum 5-HT levels were associated with clinical severity and pulmonary function in human asthma, and were increased in asthma attacks [53]. However, a contribution of elevated serum 5-HT to PH and right heart failure in acute severe human asthma is currently not known.

In conclusion, the present authors have shown that allergen sensitisation and inhalative challenge of mice, which is well established to induce allergic lung inflammation and airway hyperresponsiveness, results in distinct vascular hyperresponsiveness to different vasoconstrictive agonists. These results suggest that allergic inflammation of the lung may play a role in the pathobiology of pulmonary hypertension.

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