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Effects of arachidonic acid metabolism on hypoxic vasoconstriction in rabbit lungs

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Abstract

Hypoxic pulmonary vasoconstriction is an essential mechanism that matches lung perfusion to ventilation, thus optimising pulmonary gas exchange. Despite its pathophysiological relevance, the mechanism of hypoxic pulmonary vasoconstriction still remains enigmatic. We investigated whether arachidonic acid metabolism is involved in the regulation of hypoxic pulmonary vasoconstriction in isolated, buffer-perfused rabbit lungs. Seven inhibitors were employed to determine the contribution of different vasoactive lipoxy- and cyclooxygenase mediators as well as cytochrome P_{450} products on the magnitude of hypoxic pulmonary vasoconstriction. Hypoxic pulmonary vasoconstriction was not affected by (i) the cyclooxygenase inhibitor acetylsalicylic acid, (ii) the thromboxane A_2 receptor antagonist BM13.505, (iii) the 5′-lipoxygenase inhibitor MK886, and (iv) the lipoxygenase and cyclooxygenase inhibitor BW755c. The hypoxia-elicited pressor response was prominently inhibited by (i) nordihydroguaiaretic acid (50–150 μM), an inhibitor of lipoxygenase and cyclooxygenase and (ii) methoxsalen (100 μM) and 1-aminobenzotriazole (1–10 mM), two inhibitors of cytochrome P_{450}-derived metabolites. However, no specificity for the regulation of hypoxic pulmonary vasoconstriction was found, as corresponding inhibitory potency of these agents was noted when vasoconstriction was achieved by the stable thromboxane analogue U46619 under conditions of normoxia. We conclude that there is no evidence for a specific involvement of different pathways of arachidonic acid metabolism in the mechanism of hypoxic pulmonary vasoconstriction in rabbits.

Keywords: Arachidonic acid; Cyclooxygenase; Cytochrome P_{450}; Hypoxic pulmonary vasoconstriction; Lipoxygenase

1. Introduction

Hypoxic pulmonary vasoconstriction is an essential mechanism matching perfusion to ventilation, thus optimizing pulmonary gas exchange (for review, see Fishman, 1976; Voelkel, 1986; Marshall, 1990; Hampl and Herget, 1991). Since the first description by Von Euler and Liljestrand (1946), this field of research has attracted the attention of many scientists because of its broad clinical implication. Nevertheless, the cell(s) responsible for O_2 sensing, the sensor mechanism(s), and the molecular effectors that induce vascular smooth muscle contraction remain enigmatic. We and other investigators recently proposed that nitric oxide and a NAD(P)H oxidase are specifically involved in the regulation of hypoxic pulmonary vasoconstriction (Archer et al., 1989; Thomas III et al., 1991; Youngson et al., 1993; Mohazzab-H and Wolin, 1994; Grimminger et al., 1995a; Grimminger et al., 1995b). In addition, it has long since been suggested that changes in the balance between vasodilatory and vasoconstrictive metabolites of arachidonic acid might contribute to hypoxic pulmonary vasoconstriction. Studies in this field have, however, at least given partly contradictory results. Whereas most of the investigators have denied a specific role of arachidonic acid metabolites in hypoxic pulmonary vasoconstriction, others have suggested a significant contribution to the mechanism of hypoxic pulmonary vasoconstriction (Marshall et al., 1987; Raj and Chen, 1987; Yuan et al., 1995). Different experimental settings and species differences may have led to these conflicting results, and non-specific effects were often not discriminated from a specific efficacy on hypoxic pulmonary vasoconstriction when employing different inhibitory agents.

In the present study we addressed the effects of seven inhibitors of the different arachidonic acid metabolism

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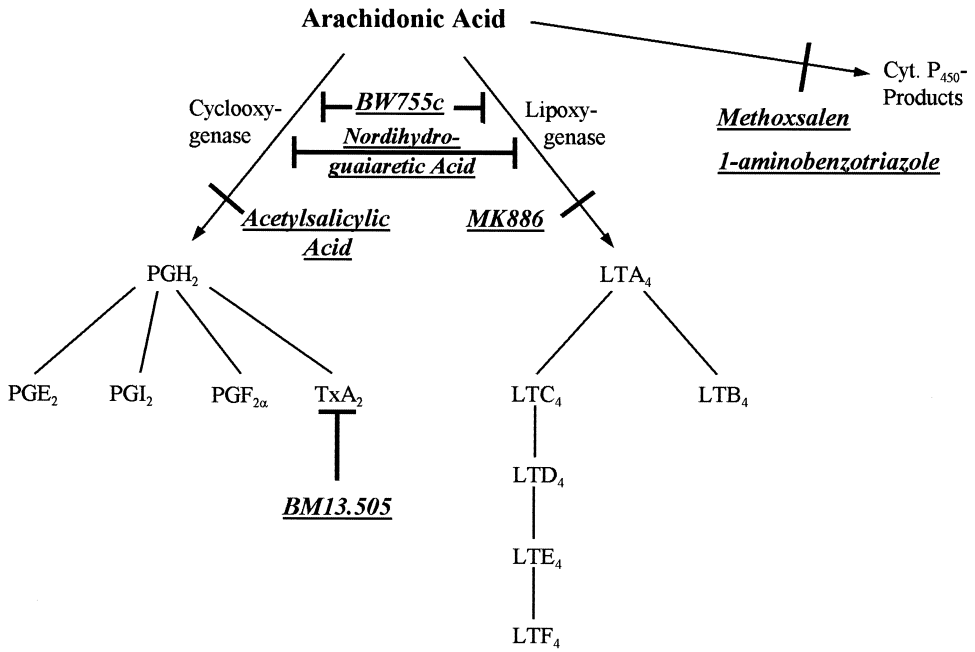


Fig. 1. Schematic presentation of the site of action of the different inhibitors of arachidonic acid metabolism. The synthesis of prostaglandins and thromboxane via cyclooxygenase and of leukotrienes via lipoxygenase, and the generation of cytochrome P_{450}-derived products is depicted. PG = prostaglandin, LT = leukotriene, Tx = thromboxane.

pathways (Fig. 1) on hypoxic pulmonary vasoconstriction in buffer-perfused rabbit lungs. If an inhibitor affected the strength of hypoxic pulmonary vasoconstriction, the specificity of this effect was tested in corresponding experiments with pharmacologically induced vasoconstriction under conditions of normoxia. The following agents were used: (i) acetylsalicylic acid, an inhibitor of cyclooxygenase, (ii) BM13.505, a thromboxane A_2-receptor antagonist, (iii) MK886, a 5′-lipoxygenase inhibitor, (iv) BW755c and nordihydroguaiaretic acid, two inhibitors of lipoxy- and cyclooxygenase and (v) 1-aminobenzotriazole and methoxsalen, two inhibitors of arachidonic acid metabolism via cytochrome P_{450}. Overall, no evidence for a specific involvement of different pathways of arachidonic acid metabolism in the regulation of hypoxic pulmonary vasoconstriction was obtained.

2. Materials and methods

2.1. Reagents

BM13.505 was obtained from Boehringer (Mannheim, Germany) and acetylsalicylic acid from Bayer (Leverkusen, Germany). MK886 was a gift from Merck Frosst (Montreal, Canada) and BW755c was provided by Wellcome (Kent, UK). Nordihydroguaiaretic acid and 1-aminobenzotriazole were purchased from Sigma (Deisenhofen, Germany). Methoxsalen was purchased from Basotherm (Biberach, Germany) and the solvent was a kind gift from Basotherm. U46619 (stable thromboxane analogue) was obtained from Paesel + Lorei (Frankfurt, Germany). All other biochemicals were purchased from Merck (Munich, Germany).

2.2. Lung isolation, perfusion, and ventilation

The model of isolated perfused rabbit lungs has been described previously (Seeger et al., 1994; Weissmann et al., 1995). Briefly, pathogen-free rabbits of either sex (body weight 2.2–3.2 kg) were deeply anesthetised by combined intravenous application of ketamine (30–50 mg/kg) and xylazine (6–10 mg/kg) and anticoagulated with heparin (1000 U/kg body weight). The lungs were excised while being perfused with Krebs Henseleit buffer through cannulae in the pulmonary artery and the left atrium. The buffer contained 125.0 mM NaCl, 4.3 mM KCl, 1.1 mM KH_2PO_4, 2.4 mM $CaCl_2$ and 1.3 mM $MgCl_2$ and 275 mg glucose per 100 ml; $NaHCO_3$ was adjusted to a constant pH range of 7.37–7.40. After rinsing the lungs with at least 1 l of buffer fluid for washout of blood, the perfusion circuit was closed for recirculation (total system volume 350 ml). Meanwhile, the flow was slowly increased from 20 to 150 ml/min, and left atrial pressure was set at 1.5–2.0 mmHg to ensure zone III conditions throughout the lung at end expiration. The alternate use of two separate perfusion circuits allowed repeated exchange of buffer fluid. In parallel with the onset of artificial perfusion, ventilation was changed from room air to a mixture of 5.3% CO_2, 21% O_2, balance N_2

(tidal volume, 30 ml; frequency, 30 strokes/min). A positive end-expiratory pressure of 1 cmH_2O was chosen (0 referenced at the hilum). The isolated, perfused lungs were placed in a temperature-equilibrated housing chamber, freely suspended from a force transducer for continuous monitoring of organ weight. The whole system (perfusate reservoirs, tubing, housing chamber) was heated to 38.5°C, ascertained by a thermistor in the pulmonary artery catheter. Pressures in the pulmonary artery, the left atrium and the trachea were registered by means of small-diameter tubing threaded into the perfusion catheters and the trachea and connected to pressure transducers. Lungs included in the study were those that (i) had a homogeneous white appearance with no signs of hemostasis, edema or atelectasis, (ii) revealed constant mean pulmonary artery and peak ventilation pressure in the normal range, and (iii) were isogravimetric during an initial steady state period of at least 20 min.

2.3. Hypoxic manoeuvres and pharmacological challenges

The technique of sequential hypoxic manoeuvres in buffer-perfused rabbit lungs has been described previously (Weissmann et al., 1995). A gas mixing chamber (KM 60-3/6MESO, Witt, Witten, Germany) was employed for step changes in the ventilator O_2 content (21% vol/vol (alveolar PO_2 ~ 160 mmHg, baseline conditions) to 3% vol/vol (alveolar PO_2 ~ 23 mmHg, hypoxic conditions)). The 5.3% vol/vol CO_2 was used throughout, and the percentage of N_2 was balanced accordingly. Buffer returning from the perfusate reservoir to the lungs passed through a membrane oxygenator (M8Exp, Jostra, Hirrlingen, Germany). By this device the partial pressure was set at ~ 40 mm Hg for both CO_2 and O_2 in the post-oxygenator buffer fluid entering the pulmonary artery. Sequential hypoxic manoeuvres of 10-min duration, interrupted by 15-min periods of normoxia, were performed. The effect of the metabolic inhibitors on pressure responses provoked by alveolar hypoxia (3% O_2) was determined within such a sequence of hypoxic manoeuvres. Each inhibitor was added to the buffer fluid 5 min prior to a hypoxic challenge, starting the addition after accomplishing the second hypoxic manoeuvre. Cumulative dose–effect curves were established. If there was a significant influence on the strength of hypoxic pulmonary vasoconstriction, the effect of each inhibitor on the hypoxia-evoked vasoconstriction was compared to its efficacy on U46619-elicited pressor responses in separate experiments. For this purpose, a mode of repetitive bolus application of this stable thromboxane analogue was used (added to the perfusate at 0.5 nM every 25 min).

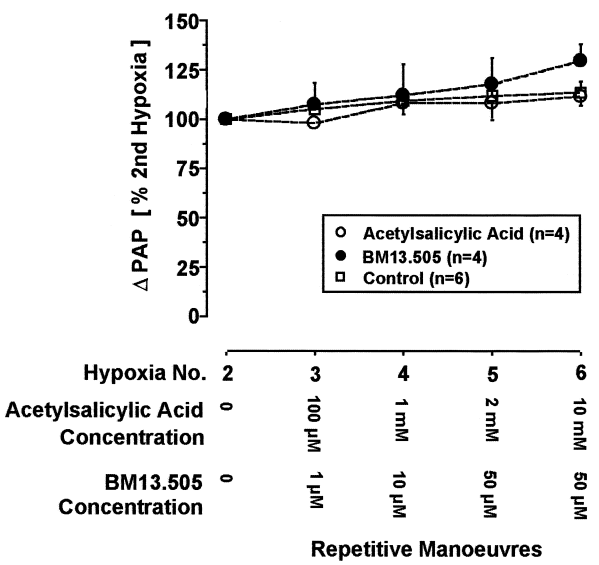


Fig. 2. Effects of acetylsalicylic acid and BM13.505 on hypoxic pulmonary vasoconstriction. The strength of the hypoxia-elicited increase in pulmonary artery pressure (ΔPAP) is given in percent (means ± S.E.M.) of the second hypoxic challenge (= reference response). Cumulative dose–effect curves are displayed. *n*, Number of lung experiments.

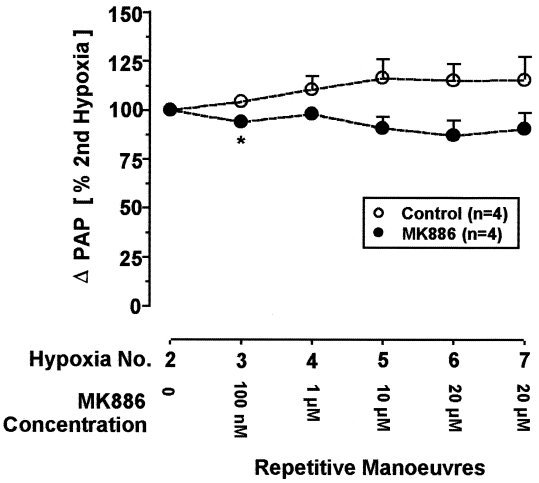


Fig. 3. Influence of MK886 on the strength of hypoxic pulmonary vasoconstriction. MK886 showed a slight tendency to inhibit hypoxic pulmonary vasoconstriction, which was, however, significant only at 100 nM (cumulative concentrations are given). The strength of the hypoxia-elicited increase in pulmonary artery pressure (ΔPAP) is given in percent (means ± S.E.M.) of the second hypoxic challenge (= reference response). Asterisk indicates significant difference by comparison with control. *n*, Number of lung experiments.

In each lung preparation, the response to the second vasoconstricting challenge was set at 100% (= reference response); the values of the following vasoconstrictions were related to this response. Control experiments were performed with use of the inhibitor vehicle only.

2.4. Statistics

Data are means ± S.E.M. For comparison of more than two groups a one-way analysis of variance with the modlsd post-test was performed. An unpaired two-tailed *t*-test was used if two groups were compared. The statistical analysis was done with SPSS/PC + 4.0 (SPSS, Chicago, IL, USA).

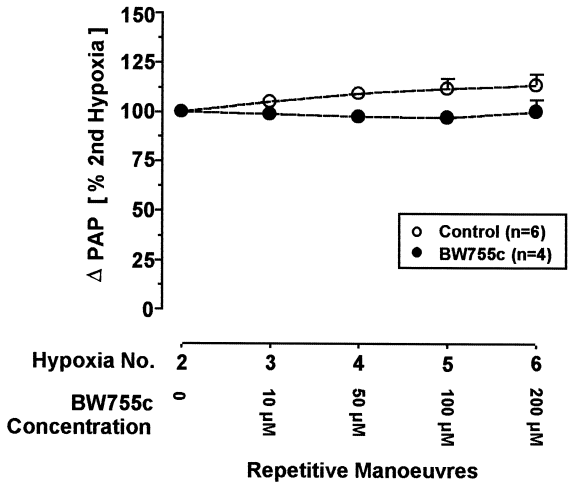


Fig. 4. Effects of BW755c on hypoxic pulmonary vasoconstriction. BW755c did not affect the strength of hypoxic pulmonary vasoconstriction. The strength of the pressor responses is given in percent (means ± S.E.M.) of the second hypoxic challenge (= reference response). Cumulative dose–effect curves are displayed. *n*, Number of lung experiments.

Statistical significance was assumed when P ranged ≤ 0.05.

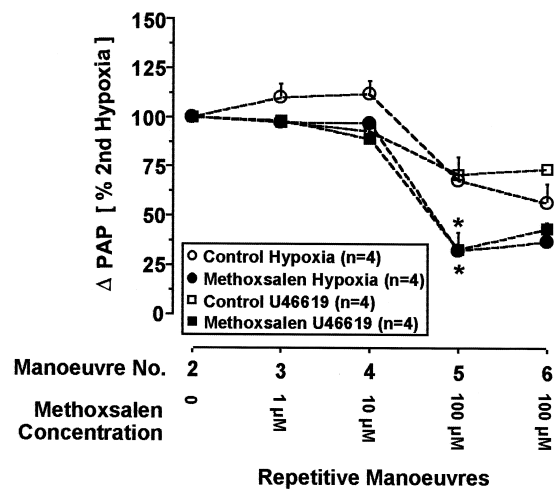
3. Results

Under baseline conditions, pulmonary artery pressure values ranged between 3.5 and 8.0 mm Hg in all experiments. A 3% hypoxic challenge (alveolar PO_2 ~23 mmHg) provoked a rapid increase in pulmonary artery pressure, with maximum pressure elevations for the reference response of 3.2 ± 0.2 mmHg (means ± S.E.M.). The hypoxia-induced vasoconstrictive manoeuvres were readily reproducible within the same lung.

The cyclooxygenase inhibitor acetylsalicylic acid was investigated in a concentration range between 100 μM and 10 mM. Even the highest dosage had no effect on the strength of hypoxic pulmonary vasoconstriction, compared to the time-matched controls (Fig. 2).

The thromboxane receptor antagonist BM13.505 did not significantly affect the reaction of the hypoxia-induced vasoconstriction in the doses of 1, 10 and 50 μM (Fig. 2) in comparison to the control.

Addition of 100 nM up to 20 μM of the 5′-lipoxygenase-inhibitor MK886 slightly diminished hypoxic



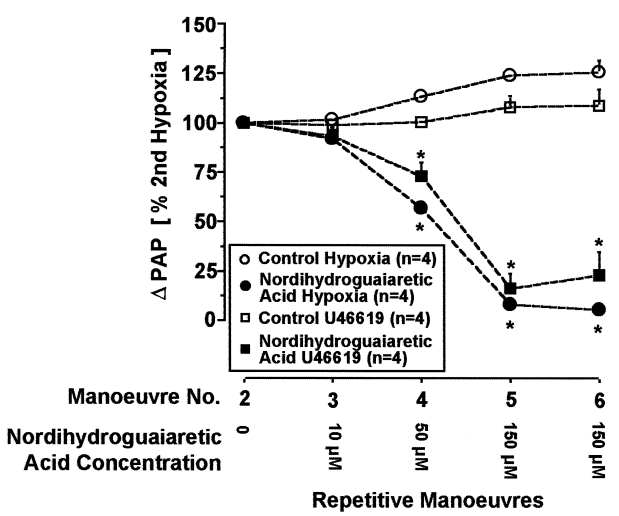


Fig. 5. Inhibition of hypoxic pulmonary vasoconstriction by nordihydroguaiaretic acid. Nordihydroguaiaretic acid inhibited hypoxic pulmonary vasoconstriction in a dose-dependent manner (cumulative concentrations are given) with nearly complete blockage at 150 μM. The strength of hypoxic pulmonary vasoconstriction is depicted in percent of the second challenge (= 100%). U46619 induced vasoconstrictions showed an identical inhibition profile. Data are given as means ± S.E.M. Asterisks indicate significant differences by comparison with the control experiments. *n*, Number of lung experiments.

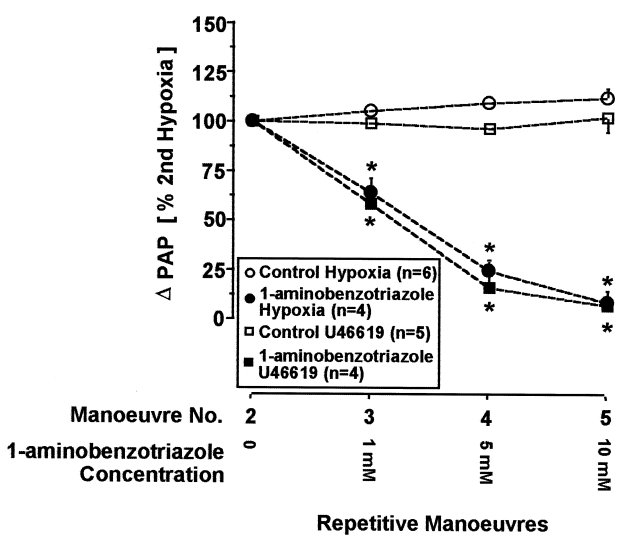


Fig. 6. Effects of methoxsalen and 1-aminobenzotriazole on hypoxic pulmonary vasoconstriction. Both methoxsalen (top) and 1-aminobenzotriazole (bottom) diminished hypoxic pulmonary vasoconstriction significantly, compared to control experiments in the absence of inhibitor, as indicated by asterisks. However, U46619-induced vasoconstrictions were significantly inhibited. Data are given in percent of the second challenge (= 100%) as means ± S.E.M. *n*, Number of lung experiments.

pulmonary vasoconstriction at 100 nM, however, this effect was not significant throughout the entire concentration range (Fig. 3).

BW755 (10–200 μM), an inhibitor of both cyclooxygenase and lipoxygenase, had no influence on the strength of hypoxia-induced vasoconstriction as compared to the control experiments (Fig. 4).

The cyclooxygenase and lipoxygenase inhibitor nordihydroguaiaretic acid dose dependently suppressed the strength of hypoxic pulmonary vasoconstriction. A concentration of 150 μM sufficed for virtually complete blockage of the hypoxia-induced vasoconstriction. However, time-matched comparison with U46619-induced vasoconstrictions showed a similar suppression of these pharmacologically induced vasoconstrictions (Fig. 5).

Both methoxsalen and 1-aminobenzotriazole showed significant inhibition of hypoxic pulmonary vasoconstriction. Methoxsalen was used in a concentration range of 1–100 μM, and 1-aminobenzotriazole was investigated between 1 mM and 10 mM. Again, no specificity for hypoxic pulmonary vasoconstriction could be demonstrated for these agents compared to the effects on U46619-induced vasoconstrictions (Fig. 6).

4. Discussion

The present study employed the model of isolated blood- and cell-free perfused rabbit lung to investigate the effects of seven inhibitors of arachidonic acid metabolism on hypoxic pulmonary vasoconstriction. In this model natural cell-to-cell contacts and local physiological regulations are maintained, but the influence of plasma-borne mediators, the central nervous system, and circulating cells can be excluded. The PO_2 and PCO_2 values in the buffer fluid entering the lung were kept constant by use of a membrane oxygenator in the perfusion circuit. Excellent reproducibility of both hypoxia- and pharmacologically (U46619) induced vasoconstriction was observed in this model (Weissmann et al., 1995, 1998).

The impact of arachidonic acid metabolites in the regulation of the hitherto unsolved regulation of hypoxic pulmonary vasoconstriction has been discussed extensively (Morganroth et al., 1984; Marshall et al., 1987; Raj and Chen, 1987; McCormack and Paterson, 1989; Pearl and Prielipp, 1991; Chang et al., 1992; Schnader et al., 1993; Yuan et al., 1995). Many investigators excluded an involvement of cyclooxygenase and lipoxygenase in the regulation of hypoxic pulmonary vasoconstriction. However, only few studies have addressed the possible involvement of cytochrome P_{450} products (Sylvester and McGowan, 1978; Miller and Hales, 1979; Knoblauch et al., 1981; Chang et al., 1992; Yuan et al., 1995), and to our knowledge no studies exist where all branches of arachidonic acid pathways were investigated in a single experimental setting. Furthermore, it has been documented that great variations may exist among different species and experimental settings concerning this pathway (Chang et al., 1984; Marshall et al., 1987; Schnader et al., 1993); e.g., results could differ whether performed in whole animals or isolated lungs either perfused with blood or salt solutions. Therefore, we used well-characterised inhibitors to block selectively the different branches of arachidonic acid pathway in blood-free perfused rabbit lungs (Letteron et al., 1986; Gottlieb et al., 1988; Chang et al., 1992; Tanaka et al., 1992; Schnader et al., 1993; Walmrath et al., 1993). In this model confounding effects of blood are excluded due to the perfusion with Krebs Henseleit buffer.

Only the two cytochrome P_{450}-inhibitors and nordihydroguaiaretic acid showed significant diminution of hypoxic pulmonary vasoconstriction. However, no specificity for hypoxic pulmonary vasoconstriction could be found by comparison with U46619-induced vasoconstriction under conditions of normoxia.

Our results thus provide no evidence for an involvement of cyclooxygenase products in the regulation of hypoxic pulmonary vasoconstriction in rabbits. Even the blockage of the thromboxane receptor had no effect on hypoxic pulmonary vasoconstriction, thus excluding an antagonism between vasodilative (e.g., prostaglandin I_2) and vasoconstrictive (predominantly thromboxane A_2) metabolites of the cyclooxygenase pathway in the regulation of hypoxic pulmonary vasoconstriction. Additionally BW755c, an unspecific lipoxygenase inhibitor with cyclooxygenase-inhibitory capacity (Garrett et al., 1987; Gottlieb et al., 1988), did not affect hypoxic pulmonary vasoconstriction, thus further supporting the results of acetylsalicylic acid addition. The second combined lipoxygenase and cyclooxygenase inhibitor, nordihydroguaiaretic acid, diminished hypoxic pulmonary vasoconstriction. This effect was, however, not specific for hypoxia-induced vasoconstriction, as the U46619-induced vasoconstriction was affected with superimposable dose-inhibition characteristics. The unspecific effect of nordihydroguaiaretic acid on vascular smooth muscle tone may be attributed to its side effects on mitochondrial energy transfer and actin–myosin filaments as discussed by Gottlieb et al. (1988) for pressor responses in isolated ferret lungs.

MK886, by specifically blocking 5′-lipoxygenase-activating protein (Dixon et al., 1990), only slightly affected the strength of the hypoxia-induced vasoconstriction at 100 nM with the tendency of marginal inhibition at the higher concentrations. However, regarding the results with BW755c, nordihydroguaiaretic acid, and MK886 a prominent role of leukotrienes in the regulation of hypoxic pulmonary vasoconstriction is apparently highly unlikely, at least in rabbit lungs. It is thus concluded that an antagonism between vasodilative and/or vasoconstrictive products of lipoxygenase or cyclooxygenase does not significantly contribute to the regulation of hypoxic pulmonary vasoconstriction in rabbit lungs, as previously

proposed for isolated perfused lamb lungs (Raj and Chen, 1987).

In a recent study, Yuan et al. (1995) provided evidence for the hypothesis that cytochrome P_{450} systems can act as oxygen sensors in hypoxic pulmonary vasoconstriction. However, the present study showed that neither the cytochrome P_{450}-inhibitor methoxsalen nor 1-aminobenzotriazole inhibited hypoxic pulmonary vasoconstriction specifically. In pilot experiments (data not shown), these inhibitors where ascertained to suppress the generation of the cytochrome P_{450}-products 8,9-epoxyeicosatrienoic acid and 14,15-epoxyeicosatrienoic acid in the perfused rabbit lungs. Thus, cytochrome P_{450}-products are unlikely candidates for the regulation of hypoxic pulmonary vasoconstriction, at least in this species. This is in line with the findings of Chang et al. (1992) in isolated perfused rat lungs.

It has to be kept in mind that our studies were performed in blood-free perfused isolated lungs. Possibly part of the conflicting results concerning the regulation of hypoxic pulmonary vasoconstriction with respect to arachidonic acid metabolites are (besides of species differences) related to interference with blood or blood-borne mediators. For example, nordihydroguaiaretic acid has been shown to amplify hypoxic pulmonary vasoconstriction in blood-perfused lungs (Brashers et al., 1988) which is in contrast to our investigation. Thus results from other studies may differ from observations of the present investigation if blood is used as the perfusate instead of a salt solution. A review of the literature does, however, not show systematic differences between blood and blood-free perfused isolated lungs with respect to arachidonic acid metabolite effects. However, the experimental setting used in the present study has the advantage that the basic mechanism of hypoxic pulmonary vasoconstriction can be investigated without interference resulting from blood components.

In conclusion, by employing inhibitors of all major pathways of arachidonic acid metabolism, no evidence for a specific role of arachidonic acid-derived lipid mediators in the regulation of hypoxic pulmonary vasoconstriction was obtained.

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