Role of arachidonic acid-derived metabolites in the control of pulmonary arterial pressure and hypoxic pulmonary vasoconstriction in rats

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Key points
• In the presence of hypoxia, vasoconstriction of pulmonary arteries occurs to avoid V/Q mismatch and maintain ventilation.
• Arachidonic acid metabolites may be involved in this process.
• Thromboxane appeared to be crucial for constriction under hypoxic conditions in isolated rat pulmonary arteries.
• This study increases our understanding of the mechanisms of hypoxic vasoconstriction.

Background. The roles of arachidonic acid (AA) metabolites in hypoxia-induced pulmonary vasoconstriction (HPV), a critical physiological mechanism that prevents ventilation/perfusion mismatch, are still incompletely understood.

Methods. Pulmonary arterial pressure was measured in ventilated/perfused rat lungs. Isometric tones of rat intralobar pulmonary arteries were also measured, using a myograph.

Results. Hypoxia (PO2, 3%)-induced pulmonary arterial pressure increases (ΔPAPhypox) were stable with blood-mixed perfusate, but decayed spontaneously. ΔPAPhypox was inhibited by 29%, 16%, and 28% by the thromboxane A2 (TXA2) antagonist SQ-29548, the 5-lipoxygenase inhibitor, MK886, and the leukotriene D4 antagonist, LY-171883, respectively. The prostacyclin synthase inhibitor tranylcypromine augmented ΔPAPhypox by 5%, whereas inhibition of cytochrome P450 did not affect ΔPAPhypox. Consistently, the TXA2 analogue U46619 increased ΔPAPhypox whereas prostacyclin abolished ΔPAPhypox. However, leukotriene D4 had no direct effect on ΔPAPhypox. In the isolated pulmonary arteries, pretreatment with U46619 was essential to demonstrate hypoxia-induced contraction.

Conclusions. The above results suggest that TXA2 and cysteinyl leukotrienes, other than leukotriene D4, are endogenous factors that facilitate HPV in rats. The indispensable role of TXA2-induced pretone in the HPV of isolated pulmonary arteries indicates that the signal from thromboxane receptors might be a critical component of oxygen sensation mechanisms.

Keywords: hypoxia; lung, blood flow; rat

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Hypoxia-induced pulmonary vasoconstriction (HPV) is critical for ventilation/perfusion equilibrium in the lungs.1–3 On the basis of in vitro studies of isolated pulmonary arteries, pulmonary arterial smooth muscle cells are believed to play a role as both sensors and effectors in HPV.4,5 However, the requirement of pretone agents to evoke HPV in isolated pulmonary arteries (see below) suggests the critical role(s) of diffusible extracellular factors. Furthermore, in experiments with ventilated/perfused (V/P) lungs, blood-mixed perfusate provides more stable HPV responses than physiological salt solution only perfused conditions.5 Metabolites of arachidonic acids (AAs) have been suggested to be candidate blood-borne modulators, because phospholipase A2 (PLA2) can be activated by hypoxia.7–10 AAs are metabolized by: (i) cyclooxygenase-synthesizing prostacyclin (PGI2) and thromboxane A2 (TXA2), (ii) lipoxygenase-synthesizing leukotrienes (LT), and (iii) cytochrome P450 (CYP) enzymes that produce epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). Previous studies on the putative roles of AA metabolites are extensive but have produced conflicting results regarding the roles of endogenous TXA2,11 LT,12–14 and EET,15–17 in HPV. A partial constriction (‘pretone’ state) is generally required to demonstrate HPV in isolated PAs.15–19 TXA2, LTs, and/or eicosanoids might be permitting factors in vivo. However, these studies were performed under different experimental conditions and in different animal species. In this respect, a systemic and comprehensive comparison under the same
Experimental conditions was required to elucidate the relative role(s) of AA metabolites in HPV. Given this background, we investigated the relative contributions made by AA metabolites to the HPV of rats.

**Methods**

**PAP measurement in V/P lungs**

This investigation was performed in accord with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996), and with the Seoul National University College of Medicine guidelines for the care and use of animals. Male Sprague–Dawley rats (250–300 g) were anaesthetized with pentobarbital sodium (40 mg kg⁻¹, i.p.). Within 5–10 min after the injection, rats fell down. Adequate anaesthesia was confirmed by two steps: (i) no reflexive eye blinking in response to touching the cornea, and then (ii) no pedal withdrawal reflex in response to toe pinching. Tracheal intubation was then performed, and the rats’ lungs were ventilated (Ventilator 683, Harvard Apparatus, USA) with normoxic gas (21% O₂, 5% CO₂, 74% N₂). Stable ventilation (85 bpm) with a regular tidal volume (10 ml kg⁻¹) was maintained. After median sternotomy and injection of heparin (200 U kg⁻¹), cannulations were made into the pulmonary artery via the right ventricle and into the left atrium via the left ventricle. The isolated lungs were then perfused at a constant flow of 15 ml min⁻¹ (Servo amplifier 2990, Harvard Apparatus). It took <10 min from confirming the full anaesthetic state to the completion of aortic cannulation. The recycling perfusate consisted of 20 ml of whole blood and 30 ml of PSS. Pulmonary arterial pressure was measured at the inflow cannula and digitally stored using Powerlab/4ST and Chart5 (AD Instruments, Sydney, Australia). Before experiments, angiotensin II (1 μg) was injected into the circuit. After restabilizing PAP (20–30 min after the angiotensin II injection), lungs were exposed to cycles of normoxia (5 min) and hypoxia (5 min) by ventilating with gas containing 21% or 3% O₂ with the balance made up with nitrogen.

**Measurement of the isometric contraction of pulmonary arteries**

After confirming adequate anaesthesia, rats were killed by decapitation. The second- and third-order branches of pulmonary arteries (ID 200–300 μm) were dissected and cut into segments (2 mm in length), and mounted on two 25 μm wires in a Mulvany-type myograph (Myo-Interface Model 410A, DMT, Denmark). A basal tone (~0.5 g) was applied in physiological salt solution (PSS), continuously gassed with 74% N₂, 21% O₂, and 5% CO₂ at 37°C.

**Solutions and chemicals**

The PSS used for the V/P lung experiment consisted of (in mM) NaCl 131, KH₂PO₄ 1.2, NaHCO₃ 22.6, CaCl₂ 3.2, MgSO₄ 1.2, glucose 11, and bovine serum albumin (BSA) 30.0 g litre⁻¹. The PSS used in the myograph experiment with pulmonary arteries consisted of (in mM): NaCl 118, KCl 4, NaH₂PO₄ 0.44, NaHCO₃ 24, CaCl₂ 1.8, MgSO₄ 1, and glucose 5.6. Isotonic high K⁺ solutions (80 mM) were prepared by replacing NaCl with an equimolar amount of KCl. BSA was purchased from Tocris (Ellisville, MO, USA). Tranilcyprone,
SQ-29548, and PGI$_2$ were purchased from Biomol (Plymouth Meeting, PA, USA). All other drugs were obtained from Sigma (St Louis, MO, USA).

Statistical analysis
The data are presented as original recordings and bar graphs of median and inter-quartile ranges (IQR) or mean and SD, as appropriate. A one-way analysis of variance (ANOVA) and the Bonferroni multiple range tests were used for the statistical analysis. Statistical significance was accepted for a $P$-value of $<0.05$.

Results
In the V/P lungs of rats, both basal PAP and hypoxia-induced change in PAP ($\Delta$PAP$_{hypox}$) decayed spontaneously when perfused with PSS-only, and these reductions were recovered by adding either whole blood or RBCs (Fig. 1). Blood-containing PSS perfusate was used in subsequent experiments. In general, HPV responses ($\Delta$PAP$_{hypox}$) were consistently observed for cyclooxygenase (COX) inhibitors (10 $\mu$M indomethacin and 5 $\mu$g ml$^{-1}$ sodium mCelofenamate, Fig. 2A). In the absence of a COX inhibitor, $\Delta$PAP$_{hypox}$ became more attenuated by repetitive hypoxia (Fig. 2A and C, n=5).

Subsequently, without COX inhibitors in the perfusate, we investigated the roles of intrinsic AA metabolites using specific receptor antagonists for the TXA$_2$ or PGI$_2$, or synthase inhibitors for leukotrienes or eicosanoids. SQ-29548 (1 $\mu$M; a selective antagonist of TXA$_2$ receptor) decreased $\Delta$PAP$_{hypox}$ by 29 (7.1)% ($P<0.05$, n=5, Fig. 3A), but $\Delta$PAP$_{hypox}$ recovered spontaneously after the third episode of hypoxia. Since LTD$_4$ has been suggested to be a modulator of pulmonary vasoconstriction, we tested the effect of the LTD$_4$ antagonist, LY-171883 (1 $\mu$M), and this was found to slightly decrease the following $\Delta$PAP$_{hypox}$ by 16 (11.1)% ($P<0.05$, n=5, Fig. 3B). MK886, a lipooxygenase inhibitor, also attenuated $\Delta$PAP$_{hypox}$ by 28 (4.2)% ($P<0.05$, n=5, Fig. 3C). In contrast, pretreatment with tranylcypromine (a PGI$_2$ synthase inhibitor) slightly increased $\Delta$PAP$_{hypox}$ by 5 (5.8)% ($P<0.05$, n=5, Fig. 3D). However, 10 $\mu$M of 17-octadecynoic acid (17-ODYA; an inhibitor of CYP-450) did not affect $\Delta$PAP$_{hypox}$ (n=5, Fig. 3E). In the above experiments, baseline PAP was...
Fig 3 Roles of endogenous TXA₂, LTD₄, PGI₂, and epoxyeicosanoids on HPV in V/P lungs. Without COX inhibitors, SQ-29548 (A), LY-171883 (B), MK-886 (C), tranylcypromine (D) and ODYA (E) were applied before the second hypoxic challenge (downward arrows). \( \Delta \text{PAP} \) values are summarized in the right panels (median, IQR, A – E). Baseline PAP (BLPAP) normalized vs controls (mean and SD) are summarized in (F). Closed bar= control, green bars with left diagonal lines= maximum changes in BLPAP after drug treatment, pink bars with right diagonal lines= BLPAP after three hypoxic treatments. *\( P < 0.05 \).
transiently decreased by SQ-29548 or LY-171883, increased by tranylcypromine, and unaffected by 17-ODYA (Fig. 3F).

We also confirmed that another type of CYT-450-dependent epoxygenase inhibitor (PPOH) had no effect on baseline PAP or D_PAPhypox (data not shown).

Next, using COX inhibitors, we tested the effects of U46619 (a TXA2 analogue), PGI2, and LTD4 on PAP and D_PAPhypox (Fig. 4). U46619 (0.2 μg) increased basal PAP by 9 (1.9)% and D_PAPhypox by 18 (6.1)% (n=6). In contrast, PGI2 (0.5 μg) markedly decreased basal PAP by 33 (1.7)% (n=3) and abolished D_PAPhypox, the latter of which recovered spontaneously. However, LTD4 (0.1 μg) changed neither basal PAP nor D_PAPhypox (n=5).

The persistent positive effects of TXA2 shown in V/P lungs were also observed in isolated pulmonary arteries. Unlike V/P lungs, hypoxia alone could not induce PA contraction (n=6, Fig. 5A), and whereas pretreatment with angiotensin II (100 nM) induced a transient contraction, no HPV was observed (n=7, Fig. 5A). In contrast, at a relatively low concentration of U46619 (10 nM), a robust HPV was consistently observed [95 (4.9)% of 80 K+ contraction, n=13, Fig. 5A and C]. However, 10 nM U46619 alone induced only a slight contraction; 5–10% of 80 K+ contraction (Fig. 5C). U46619-induced contractions and HPV were blocked by SQ-29548 (Fig. 5A and D). Indomethacin pretreatment did not affect HPV in the presence of U46619 (Fig. 5A and D).
Discussion

Our results suggest that intrinsic or blood-borne TXA$_2$ and PGI$_2$ regulate HPV in V/P lungs in a positive and negative manner, respectively. TXA$_2$ was found to be consistently indispensable to the induction of HPV in isolated pulmonary arteries. Unlike a recent report, which argued the critical role played by EET, HPV was not influenced by inhibitors of CYP-450. When comparing relative changes of D$_{PAP}$hypox induced by SQ-29548 or tranylcypromine, endogenous TXA$_2$ seemed to be more influential than PGI$_2$. However, the decaying tendency of HPV in the absence of a COX inhibitor implies a substantial influence of vasodilatory metabolites (e.g. PGI$_2$) in vivo.

TXA$_2$ is a potent vasoconstrictor of PA and also has mitogenic and platelet-aggregating properties. In addition, electrophysiological studies have demonstrated that voltage-gated K$^+$ channel currents ($I_{Kv}$) are inhibited by TXA$_2$, which might explain the positive effects on HPV. For HPV, the ‘K$^+$ channel hypothesis’ is one of the most popular theories, whereby the hypoxic inhibition of $I_{Kv}$ leads to membrane depolarization. However, considering the complexity of the suggested mechanisms of HPV, further investigation is required to elucidate the effects of TXA$_2$ on ion channels in PASMCs.

Leukotrienes were first discovered in lung perfusate and have been found to have multiple effects on the pulmonary vasculature. The inhibitory effect of MK886 on D$_{PAP}$hypox suggests a putative role for cysteinyl-leukotrienes in HPV (Fig. 3b). Actually, in a previous study, one of the authors found that LTC$_4$ increased D$_{PAP}$hypox in rats but not basal PAP. In the present study, we examined the role of LTD$_4$, because a previous study in rat lungs demonstrated a rapid conversion of LTC$_4$ into LTD$_4$. However, LTD$_4$ had no

Fig 5 Critical role of TXA$_2$ for HPV in PAs. (A) Hypoxia (P$_{O2}$, 3%) alone did not induce HPV. U46619 (10 nM) induced a slight increase in basal tone, and hypoxia (P$_{O2}$, 3%) in the presence of U46619 induced a strong contraction. Angiotensin II (30 nM) induced a transient contraction, but additional hypoxia had no effect. (b) Indomethacin (10 μM) pretreatment did not affect HPV in the presence of U46619 (10 nM, left panel), whereas SQ-29548 (1 μM) completely blocked U46619 (50 nM)-induced contraction and HPV (right panel). In (c) and (d), summaries of PA tone normalized to 80 K$^+$ contraction are shown in bar graphs (mean and sd). Indo, indomethacin; U, U46619; SQ, SQ-29548.
significant effect on PAP or $\Delta$PAP$_{hypox}$ (Fig. 4c). Overall, the present study suggests that intrinsic LTC$_4$ might facilitate HPV responses, and that the partial decrease in $\Delta$PAP$_{hypox}$ by LY-171883 might be due to the unspecific inhibition of LTC$_4$ receptors in PAs (Fig. 3e).

In the present study, we aimed to provide an understanding of the relative importance of AA metabolites in the regulation of HPV. Although the results are limited to the responses of rat lungs, of the major types of AA metabolites, TXA$_2$ appears to play a dominant role in the maintenance of PAP and HPV. In contrast, endogenous PGI$_2$ might negatively balance net HPV responses. Furthermore, the indispensable pretonic HPV responses, and that the partial decrease in $\Delta$PAP$_{hypox}$ by LY-171883 might be due to the unspecific inhibition of LTC$_4$ receptors in PAs (Fig. 3e).

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Conflict of interest
None declared.

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