Cytosolic phospholipase A2 in hypoxic pulmonary vasoconstriction

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Cytosolic phospholipase A2 (cPLA2) releases arachidonic acid (AA) from phospholipids in cell membranes. To assess the role of cPLA2 in hypoxic pulmonary vasoconstriction (HPV), we measured the increase in left lung pulmonary vascular resistance (LPVR) before and during hypoxia produced by left main stem bronchus occlusion (LMBO) in mice with and without a targeted deletion of the PLA2g4a gene that encodes cPLA2α. LMBO increased LPVR in cPLA2α+/+ mice but not in cPLA2α−/− mice. cPLA2α+/+ mice were better able to maintain systemic oxygenation during LMBO than were cPLA2α−/− mice. Administration of a cPLA2 inhibitor, arachidonyl trifluoromethyl ketone, blocked the LMBO-induced increase in LPVR in wild-type mice, while exogenous AA restored HPV in cPLA2α−/− mice. Intravenous angiotensin II infusion increased PVR similarly in cPLA2α+/+ and cPLA2α−/− mice. Inhibitors of cyclooxygenase or nitric oxide synthase restored HPV in cPLA2α−/− mice but not in cPLA2α+/+ mice. Breathing 10% oxygen for 3 weeks produced less right ventricular hypertrophy in cPLA2α−/− mice than in cPLA2α+/+ mice. Inhibitors of cyclooxygenase or nitric oxide synthase restored HPV in cPLA2α−/− mice but not in cPLA2α+/+ mice despite the continued absence of cPLA2 activity. These results indicate that cPLA2 contributes to the murine pulmonary vasoconstrictor response to hypoxia. Augmenting pulmonary vascular tone restores HPV in the absence of cPLA2 activity.


Introduction

Hypoxic pulmonary vasoconstriction (HPV) is an intrinsic property of pulmonary vascular smooth muscle that produces vasoconstriction in poorly ventilated lung regions (1). This important mechanism acts to enhance the matching of ventilation with perfusion and maintain arterial oxygenation in lung disease. Despite intensive investigation, a comprehensive understanding of the cellular mechanisms that underlie HPV remains elusive.

In the lung, the metabolism of arachidonic acid (AA) by cyclooxygenases (COX), lipoxygenases (LOs), and cytochrome P450 (CYP450) enzymes generates a wide variety of eicosanoids, including prostaglandins, thromboxanes, leukotrienes, epoxyeicosaatrienoic acids (EETs), and hydroxyeicosatetraenoic acids (HETEs), that importantly regulate vascular tone (for recent reviews see refs. 2, 3). Potential roles for eicosanoids in HPV have been studied extensively with conflicting results reported. For example, administration of exogenous AA has been shown to either attenuate (4) or enhance HPV (5). Moreover, studies of HPV using inhibitors of COX, LOs, and CYP450 enzymes are limited by the incomplete specificity of the inhibitors, as well as the metabolic diversion of AA to alternate pathways.

Unesterified AA is present at very low levels within cells, and its rate of formation generally controls the biosynthesis of bioactive eicosanoids (2). Release of AA from the sn-2 position of membrane phospholipids is mediated by phospholipase A2 (PLA2) enzymes. PLA2 enzymes can be classified into three main types based on biological properties, including secretory PLA2 (sPLA2), cytosolic PLA2 (cPLA2), and intracellular Ca2+-independent PLA2 (iPLA2). Here, we studied group IVA cPLA2, because AA generated by this PLA2 is tightly linked to the COX and LO pathways (6) and this PLA2 is known to be expressed in murine lung (7).

To assess the contribution of cPLA2 to HPV, we compared the increase in left lung pulmonary vascular resistance induced by selective left lung hypoxia in wild-type mice and cPLA2-deficient mice. Here, we report that an absence of cPLA2 activity prevents HPV in mice.

Methods

All animal experiments were conducted under protocols reviewed and approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital. Mice with a deletion of the PLA2g4a gene (cPLA2α−/−) (7) and their wild-type littermates (cPLA2α+/+) were maintained at the Massachusetts General Hospi-
Animal Resource Facility. C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). We tested mice of both sexes with an age range of 2–5 months, weighing 18–30 g. Animals in each experimental group were matched for sex and age.

**Measurement of HPV in mice.** We surgically prepared mice for hemodynamic study as described previously, and systemic artery pressure (SAP), pulmonary artery pressure (PAP), and left pulmonary artery blood flow (QLPA) were continuously recorded (8). To estimate the left lung pulmonary vascular resistance (LPVR), the inferior vena cava (IVC) was partially occluded with a circumferential 5-0 silk ligature to transiently reduce cardiac output until QLPA was reduced by approximately 50%. To calculate LPVR, the flow-pressure relationship was constructed by plotting approximately 50 consecutive digitized data points of linear parts of PAP and QLPA tracings during transient IVC occlusions. The best-fit line that describes the relationship between the slopes in each mouse (9).

Vessel morphometry. After breathing for 3 weeks at an inspired oxygen fraction (FiO2) of 0.21 (normoxia) or 0.10 (hypoxia), the airways and pulmonary vessels of cPLA2α+/− and cPLA2α+/+ mice (n= five each group) were perfusion-fixed. Lung tissue was embedded in Historesin Plus (Leica Microsystems Inc., Deerfield, Illinois, USA), and 2-µm-thick sections were stained with 0.1% toluidine blue (10). Vessels (greater than 14 and less than 100 µm in external diameter [ED]) associated with alveolar ducts or in the alveolar wall were evaluated. The percentage of wall thickness (%WT) of each transversely cut vessel was calculated (as WT × 100/ED for partially muscular and 2 × WT × 100/ED for fully muscular vessels). The endothelial component of the vessel wall was excluded from the measurement of WT.

Measurement of PL2α activity in lung homogenates. Mice were sacrificed after breathing at a FiO2 of 0.10 or 0.21 for 3 weeks, and the right lung was homogenized in ice-cold buffer containing 10 mM HEPES, 1 mM EDTA, 0.34 M sucrose, 1 µg/ml aprotinin, 1 µM pepstatin A, 1 mM PMSF, and 100 µM leupeptin. Crude homogenates were centrifuged at 4°C for 5 minutes at 5,000 g, and PL2α activity in the supernatant was measured, as described previously (11), using 1-stearyl-2-[14C] arachidonoyl-phosphatidylcholine (PC) as substrate for cPL2α and 1-stearyl-2-[14C] arachidonoyl-phosphatidylethanolamine (PE) for both sPL2α and cPL2α. To measure activity of iPL2α, 30 µg of lung protein were incubated with 50 nmol 1-palmitoyl-2-[14C] palmitoyl-phosphatidylcholine, 1 mM ATP, and 2 mM DTT in a reaction volume of 500 µl for 30 minutes at 37°C (12).

Identification of murine lung eicosanoids during HPV. At the end of HPV experiments, cPLA2α−/− and cPLA2α+/− mice were sacrificed (n= 5 each), and their hypoxic left lungs and oxygen-ventilated right lungs were rapidly excised and snap-frozen. The samples were prepared by solid-phase extraction, and liquid chromatography-tandem mass spectrometry (LC/MS/MS) was performed to characterize the eicosanoid profiles with an LCQ ion trap mass spectrometer system (Finnigan Corp., San Jose, California, USA) (13). Eicosanoids were identified by their respective MS/MS and retention times compared with those of synthetic standards. In some experiments, deuterium-labeled LTB4-d4 and thromboxane B2-d4 (Cayman Chemical, Ann Arbor, Michigan, USA) were used as internal standards. In addition, levels of prostaglandins E2 (PGE2) and F2α (PGF2α) and thromboxane B2 (TXB2) were analyzed by ELISA using commercially available ELISA kits (Neogen Corp., Lexington, Kentucky, USA).

Measurements of HPV in cPLA2α−/− mice. Measurements of LPVR were carried out in cPLA2α−/− mice (n= 10) and cPLA2α+/− mice (n= 11) before and 5 minutes after LMBO. After LPVR was measured, arterial blood was sampled by direct left ventricular puncture for blood gas analysis during LMBO.

Effects of cPLA2 inhibitor on HPV. C57BL/6 mice were treated with the selective cPLA2 inhibitor, arachidonyl trifluoromethyl ketone (ATK) (20 mg/kg dissolved in 100 µl of 2.5 vol% DMSO; n= 4) or were treated with vehicle (n= 3) by a single intravenous injection 30 minutes before measurement of △LPVR. The dose of ATK and timing of administration were chosen based on data published previously (14).

Effects of exogenous AA on HPV. The cPLA2α−/− mice (n= 6) and cPLA2α+/− mice (n= 3) received a continuous intravenous infusion of sodium salt of AA (1 µg/kg/min) for 60 minutes before measurements of △LPVR. We selected this dose of AA on the basis of results from pilot experiments.

Endothelium-dependent responses to angiotensin II. Measurements of total pulmonary and systemic vascular resistances (TPVR and TSVR, respectively) were obtained in cPLA2α−/− mice (n= 6) and cPLA2α+/− mice (n= 6) before and during an intravenous infusion of increasing doses of angiotensin II (0.05, 0.5, and 5 µg/kg/min), as described previously (8). Cardiac output was estimated by measuring lower thoracic aortic flow (QLTAF), while SAP and PAP were continuously recorded. In additional mice, the effects of ATK pretreatment (20 mg/kg) on the pulmonary vasoconstriction induced by angiotensin II infusion were examined.

Measurements of effects of COX inhibition on HPV. △LPVR was measured 30 minutes after intravenous adminis-
Hemodynamic measurements before LMBO (baseline) and 5 minutes after LMBO in cPLA2α+/− and cPLA2α−/− mice. All values at baseline before LMBO were compared between groups by ANOVA. A post hoc comparison. CP < 0.05 vs. baseline value of the same parameter in the same group. HR, heart rate.

Effects of an inhibitor of iPLA2 on HPV. ∆LPVR was measured in cPLA2α+/− (n = 3) and cPLA2α−/− (n = 3) mice that were treated with the selective iPLA2 inhibitor, bromo- moenol lactone (BEL); (Biomol Research Laboratories, Plymouth Meeting, Pennsylvania, USA; dissolved in 100 µl of 2.0 volume percent DMSO) by two intravenous injections of 0.1 mg/kg 30 minutes before and during LMBO. The dose of BEL was calculated to produce a plasma concentration of 10 µM, which was previously shown to abrogate hypoxia-induced increase in AA release from cardiac myocytes (19). Additional mice of each genotype were treated with vehicle alone (n = 3 for each genotype).

Statistical analysis. Differences between groups were determined using a two-way ANOVA. When significant differences were detected by ANOVA, a post hoc Scheffé test was employed (Statistica for Windows; StatSoft, Inc., Tulsa, Oklahoma, USA). A P value less than 0.05 indicated a significant difference. All data are expressed as SEM.

Results

Unilateral alveolar hypoxia and the contribution of cPLA2. To assess the contribution of cPLA2 to HPV, we examined changes of LPVR in response to LMBO in cPLA2α+/− and cPLA2α−/− mice. Before LMBO, hemodynamic parameters did not differ between the two genotypes (Table 1). LPVR was similar in cPLA2α−/− mice (107 ± 13
Table 2

Arterial blood gas analysis

<table>
<thead>
<tr>
<th></th>
<th>cPLA2+/+</th>
<th>cPLA2–/–</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.38 ± 0.04</td>
<td>7.32 ± 0.04</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>36.3 ± 5.1</td>
<td>37.7 ± 2.9</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>298 ± 46</td>
<td>159 ± 23\[^a]</td>
</tr>
<tr>
<td>HCO3− (mmol/l)</td>
<td>20.1 ± 1.6</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.3 ± 1.4</td>
<td>13.4 ± 0.6</td>
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Arterial blood gas analyses at the end of hemodynamic studies in cPLA2+/+ mice (n = 6) and cPLA2–/– mice (n = 8) breathing FIO2 = 1 during LMBO. ^[^a]P < 0.05 vs. cPLA2+/+ mice.

mmHg/ml/min/g) and cPLA2–/– mice (97 ± 6 mmHg/ml/min/g). In cPLA2–/– mice, LMBO decreased QLPA without changing PAP, more than doubling LPVR (216 ± 29 mmHg/ml/min/g; P < 0.005; Table 1 and Figure 1a). In contrast, LMBO did not change LPVR in cPLA2+/+ mice (100 ± 10 mmHg/ml/min/g; Figure 1b), consistent with impaired HPV. In a subset of animals of each genotype (n = 3–5), QLTAF was measured as an estimate of cardiac output: LMBO did not alter QLTAF or TPVR in either genotype, demonstrating that the LMBO-induced changes in LPVR cannot be attributed to alterations in cardiac output.

To estimate the impact of impaired HPV on arterial oxygenation, we performed arterial blood gas analysis at the end of HPV measurements during LMBO while the right lung was ventilated at FIO2 = 1. Arterial partial pressure of oxygen (PaO2) was significantly higher in cPLA2–/– mice than in cPLA2+/+ mice (298 ± 46 and 159 ± 23 mmHg, respectively; P < 0.05; Table 2). There was no difference in blood pH, arterial partial pressure of carbon dioxide (PaCO2), or HCO3− (Table 2). These observations are consistent with increased intrapulmonary shunting in cPLA2–/– mice. Next, we tested whether or not pharmacological inhibition of cPLA2 impaired HPV in wild-type mice. The LMBO-induced increase in LPVR was markedly attenuated when mice were studied 30 minutes after treatment with the cPLA2 inhibitor, ATK, as compared with mice treated with vehicle alone (Figure 2). These results confirm that decreased cPLA2 activity is associated with impaired HPV in mice.

Since cPLA2 is believed to be a major enzymatic source of AA for eicosanoid biosynthesis (3, 20) in most mammalian cells, we examined whether administration of AA restores HPV in cPLA2–/– mice. After continuous intravenous infusion of AA at 1 µg/kg/min for 60 minutes, LMBO increased LPVR in cPLA2–/– mice (Figure 2). In contrast, when studied in cPLA2+/+ mice, the magnitude of the LMBO-induced increase of LPVR was not altered by exogenous AA. These results suggest that impaired HPV induced by cPLA2 deficiency was attributable to decreased availability of AA in the hypoxic lung of cPLA2–/– mice.

To examine the specificity of the effects of cPLA2 deficiency on HPV, we examined whether cPLA2 deficiency attenuates the normoxic pulmonary vasoconstrictor response to intravenous infusion of 0.05, 0.5, and 5 µg/kg/min of angiotensin II. TVPVR increased from 74 ± 12 mmHg/ml/min/g at baseline to 177 ± 18 mmHg/ml/min/g at 5 µg/kg/min angiotensin II in cPLA2+/+ mice (P < 0.01) and from 69 ± 13 to 217 ± 39 mmHg/ml/min/g in cPLA2–/– mice (P < 0.001). TSVR increased from 468 ± 66 mmHg/ml/min/g at baseline to 956 ± 216 mmHg/ml/min/g at 5 µg/kg/min angiotensin II in cPLA2+/+ mice (P < 0.01) and from 435 ± 47 to 899 ± 144 mmHg/ml/min/g in cPLA2–/– mice (P < 0.001). At each angiotensin II infusion dose, there was no difference in either the TPVVR or TSVR between cPLA2+/+ and cPLA2–/– mice. Similarly, the pulmonary vasoconstrictor response to angiotensin II did not differ between ATK-treated and vehicle-treated wild-type mice (data not shown). Taken together, our results demonstrate that cPLA2 deficiency attenuated pulmonary vasoconstriction to hypoxia, without causing nonspecific dysfunction of the vasomotor contractile apparatus.

**Pulmonary eicosanoid profiles.** To identify the major eicosanoids generated in the lungs of cPLA2+/+ and cPLA2–/– mice, we carried out profiling of eicosanoids using both ELISA and LC/MS/MS analyses. In lungs obtained from both cPLA2+/+ and cPLA2–/– mice, we identified PGE2, TXB2, 5S-HETE, 12S-HETE, and 15S-HETE by matching their respective mass spectra and retention times with those of authentic synthet-

![Figure 2](https://example.com/figure2.png)

LMBO-induced increase in LPVR is shown for cPLA2+/+ (n = 11) and cPLA2–/– (n = 10) mice at baseline and for wild-type C57BL/6 mice treated with vehicle (Veh; n = 5) or with ATK (n = 5). LMBO-induced increase in LPVR is also shown for mice during AA administration (n = 3 for cPLA2+/+ and n = 6 for cPLA2–/–), after administration of indomethacin (Indo; n = 4 for cPLA2+/+ and n = 5 for cPLA2–/–), after administration of L-NAME (n = 3 for cPLA2+/+ and n = 5 for cPLA2–/–), and after breathing at FIO2 0.10 for 3 weeks (3wk Hypoxia; n = 6 for cPLA2+/+ and n = 10 for cPLA2–/–). *P < 0.05 vs. cPLA2+/+ mice at baseline. **P < 0.05 vs. cPLA2–/– mice at baseline.
ic standards (Figure 3, a and b). Leukotriene B4 and EETs, as well as their respective omega-oxidation products, were not present in appreciable amounts in snap-frozen lungs from either genotype (Figure 3c) (2, 21). Moreover, 20-HETE was not detected in lungs of either genotype (22).

To examine the possibility that decreased levels of vasoconstrictor eicosanoids contribute to the impaired HPV in cPLA2α−/− mice, we quantitated vasoactive eicosanoids PGE2, PGF2α, and TXB2 by ELISA in cPLA2α−/− and cPLA2α+/+ mice after LMBO. PGE2 levels were higher in left than in right lungs in both genotypes (P < 0.05 for both; Figure 4a). PGF2α and thromboxane levels were greater in left than in right lungs of cPLA2α−/− mice. However, statistically significant differences were not found in the amounts of PGE2, PGF2α, or TXB2 when comparing corresponding lungs of cPLA2α+/+ and cPLA2α−/− mice.

Both indomethacin and L-NAME restored HPV in cPLA2α−/− mice. To examine the possibility that HPV is impaired in cPLA2α−/− mice due to an altered vasoconstrictor/vasodilator balance, we studied the effects of HPV in cPLA2α−/− mice breathing air (25.2% ± 1.4% and 24.2% ± 1.5%, respectively). Although prolonged hypoxia increased RV/LV+S in both genotypes, RV hypertrophy was less prominent in cPLA2α−/− mice (30.3% ± 1.4%) than in cPLA2α+/+ mice (35.5% ± 1.4%; P < 0.05). The cPLA2α−/− mice and cPLA2α+/+ mice had
and measured by ELISA. Filled bars indicate TXB₂ in either lung between the two genotypes.

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similar hemoglobin levels under normoxic conditions (12.9 ± 0.5 and 12.9 ± 0.3 g/dl, respectively). Although chronic hypoxia increased the circulating hemoglobin concentration in both genotypes, cPLA₂α+/– mice developed less polycythemia than cPLA₂α+/+ mice (16.5 ± 1.6 vs. 19.0 ± 0.3 g/dl; P < 0.05). Quantitative analysis revealed that the WT of pulmonary arterial vessels with external diameter between 14 and 100 μm was similar in cPLA₂α+/– mice and cPLA₂α+/+ mice breathing at FIO₂ 0.21 (8% ± 2% vs. 8% ± 2%). Three weeks of hypoxia increased the WT of these pulmonary vessels similarly in both groups of mice (14% ± 2% vs. 14% ± 1%). Qualitative differences in the components of the vessel walls of the two genotypes were not evident after 3 weeks of hypoxia.

Baseline PAP, QLPA, and LPVR were measured at thoracotomy in mice during ventilation at FIO₂ 1. PAP was greater in both cPLA₂α+/+ and cPLA₂α–/– mice that breathed at FIO₂ 0.10 for 3 weeks than in mice that breathed at FIO₂ 0.21 (Table 1). Breathing at FIO₂ 0.10 for 3 weeks did not increase the baseline LPVR significantly in mice of either genotype. Prolonged hypoxia did not alter the ability of LMBO to increase LPVR in cPLA₂α+/+ mice. In contrast, after 3 weeks of hypoxia, cPLA₂α–/– mice showed a robust increase of LPVR in response to LMBO, demonstrating restored HPV in cPLA₂α+/+ mice (Figure 2). Of note, after breathing at FIO₂ 0.10 for 3 weeks, cPLA₂α–/– mice had lower LPVR at baseline and during LMBO compared with cPLA₂α+/+ mice, apparently due to increased QLPA in cPLA₂α–/– mice (Table 1).

Lung homogenates obtained from normoxic cPLA₂α+/+ mice de-esterified AA from PC, whereas lung homogenates from normoxic cPLA₂α+/– mice metabolized PC only minimally (P < 0.05, Figure 5a). In contrast, lung homogenates from the normoxic cPLA₂α+/– mouse contained PLA₂ activity directed against PE in assay conditions favoring the activity of sPLA₂. PE-metabolizing PLA₂ activity was greater in cPLA₂α+/+ mice (P < 0.05 vs. cPLA₂α+/– mice; Figure 5b) most likely because cPLA₂ also acts on PE. Prolonged hypoxic exposure did not affect the activity of pulmonary cPLA₂ or sPLA₂ in either cPLA₂α+/+ mice or cPLA₂α–/– mice (Figure 5, a and b). Lung homogenates obtained from normoxic cPLA₂α+/+ mice contained iPLA₂ activity, and it was not affected by breathing at FIO₂ 0.10 for 3 weeks (Figure 5c). The iPLA₂ activity in lung homogenates obtained from normoxic cPLA₂α+/– mice was modestly greater than that in lung homogenates from cPLA₂α+/+ mice (P = 0.055), and it was decreased by breathing at FIO₂ 0.10 for 3 weeks (P < 0.05 vs. normoxic cPLA₂α+/+ mice; Figure 5c). Taken together, chronic hypoxia restored HPV in cPLA₂α–/– mice without augmenting pulmonary PLA₂ activities.

Effects of inhibition of iPLA₂ on HPV. Since pulmonary iPLA₂ activity tended to be greater in normoxic cPLA₂α+/+ mice than in cPLA₂α+/– mice, and iPLA₂ activity in cPLA₂α–/– mice was decreased by prolonged breathing at FIO₂ 0.1, we considered the possibility that compensatory upregulation of iPLA₂ in cPLA₂α+/– mice impaired HPV at baseline and reduction of iPLA₂ by chronic hypoxia restored HPV in cPLA₂α–/– mice. To test this hypothesis, we examined effects of BEL, a selective iPLA₂ inhibitor, on HPV. Administration of BEL or vehicle did not affect baseline hemodynamics (data not shown). Administration of BEL did not affect HPV in cPLA₂α+/+ mice (ΔLPVR ~ 117% ± 20% and 102% ± 18% in BEL-treated and vehicle-treated cPLA₂α+/+ mice, respectively) or in cPLA₂α–/– mice (ΔLPVR ~ 21% ± 10% and 28% ± 12% in BEL-treated and vehicle-treated cPLA₂α–/– mice, respectively; both P < 0.05 vs. cPLA₂α–/– mice with the same treatment). These results suggest that it is unlikely that a compensatory upregulation of iPLA₂ is responsible for impaired HPV in cPLA₂α–/– mice.

Discussion

The present study demonstrates that cPLA₂ deficiency impairs HPV in mice resulting in a reduced ability to preserve systemic oxygenation after LMBO. Restoration
of HPV in \textit{cPLA2}\textsuperscript{α−/−} mice with exogenous AA suggests that AA availability itself and/or one or more downstream bioactive eicosanoids contribute to HPV in wild-type mice. Although we found that \textit{cPLA2}\textsuperscript{α−/−} mice are capable of producing eicosanoids (Figure 3) and could not demonstrate quantitative differences in the levels of AA-derived eicosanoids in lungs taken from \textit{cPLA2}\textsuperscript{α−/−} and \textit{cPLA2}\textsuperscript{α+/+} mice (Figure 4), it is possible that vasoactive eicosanoids present at low levels and/or generated from a minority of cells within the lungs of \textit{cPLA2}\textsuperscript{α−/−} mice (and hence not detected in whole-lung extracts using LC/MS/MS or ELISA) may be absent from those cells in \textit{cPLA2}\textsuperscript{α−/−} mice, thereby impairing HPV.

Alternatively, deficiency of AA production in \textit{cPLA2}\textsuperscript{α−/−} mice may modulate HPV. For example, a NADPH oxidase has been implicated in the sensing of pulmonary hypoxia (23, 24). Recently, Dana et al. used a \textit{cPLA2}-deficient cell line and showed that \textit{cPLA2} and released AA are required for NADPH oxidase activation (25). It is possible that administration of AA restored HPV in \textit{cPLA2}\textsuperscript{α−/−} mice by activating NADPH oxidase-related events.

Impaired HPV in the \textit{cPLA2}\textsuperscript{α−/−} mice may be due to an alteration in the pulmonary vasoconstrictor/vasodilator balance, and administration of exogenous AA may have restored “balance.” This hypothesis is supported by our observations that both indomethacin and L-NAME restored HPV in \textit{cPLA2}\textsuperscript{α−/−} mice using doses that do not alter baseline values of PAP and QLPA. Given the increased levels of COX products we detected in the left pulmonary arteries from \textit{cPLA2}\textsuperscript{α−/−} and \textit{cPLA2}\textsuperscript{α+/+} mice (Figure 4), it is possible that vasoactive eicosanoids present at low levels and/or generated from a minority of cells within the lungs of \textit{cPLA2}\textsuperscript{α−/−} mice (and hence not detected in whole-lung extracts using LC/MS/MS or ELISA) may be absent from those cells in \textit{cPLA2}\textsuperscript{α−/−} mice, thereby impairing HPV.

Because agents that attenuate HPV can prevent pulmonary hypertension and vascular remodeling in chronically hypoxic animals (26–28), we studied the effects of chronic hypoxia on \textit{cPLA2}\textsuperscript{α−/−} and \textit{cPLA2}\textsuperscript{α+/+} mice. Breathing at F\textsubscript{O2} 0.10 for 3 weeks increased PAP similarly in both mouse genotypes. In addition, pulmonary vascular remodeling was evident in both \textit{cPLA2}\textsuperscript{α−/−} and \textit{cPLA2}\textsuperscript{α+/+} mice, and there was no difference in pulmonary vascular wall thickness between these genotypes. The observation that \textit{cPLA2} deficiency partially protects against the development of RV hypertrophy in chronically hypoxic mice is potentially attributable to multiple factors. The initial absence of HPV, as well as the more modest increase in PVR after chronic hypoxia, likely decreased the stimulus for RV hypertrophy in \textit{cPLA2}\textsuperscript{α−/−} mice. In addition, \textit{cPLA2} deficiency may have attenuated RV hypertrophy by reducing the degree of polycythemia (18).

Since \textit{cPLA2}\textsuperscript{α−/−} mice unexpectedly developed pulmonary vascular remodeling after prolonged breathing at F\textsubscript{O2} 0.10, we investigated whether prolonged hypoxic exposure restored HPV in \textit{cPLA2}\textsuperscript{α−/−} mice. In wild-type mice, prolonged breathing at F\textsubscript{O2} 0.10 did not alter the pulmonary vasoconstrictor response to LMBO. This finding in mice contrasts with previous reports suggesting that chronic hypoxia attenuates HPV in rats (29) and is possibly attributable to differences in the methods used to monitor HPV (30). In \textit{cPLA2}\textsuperscript{α−/−} mice, 3 weeks of breathing at F\textsubscript{O2} 0.10 restored the ability of LMBO to increase LPVR to the levels measured in wild-type mice without augmenting pulmonary \textit{PLA2} activities (Figure 5). It is possible that currently unappreciated compensatory mechanisms provided AA in the \textit{cPLA2}\textsuperscript{α−/−} mice after breathing at F\textsubscript{O2} 0.10 for 3 weeks,
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