Mitochondrial complex II is essential for hypoxia-induced pulmonary vasoconstriction of intra- but not of pre-acinar arteries

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Aims
Alveolar hypoxia acutely elicits contraction of pulmonary arteries, leading to a rise in pulmonary arterial pressure (PAP) and shifting blood to better ventilated areas of the lung. The molecular mechanisms underlying this hypoxic pulmonary vasoconstriction (HPV) are still incompletely understood. Here, we investigated the role of succinate dehydrogenase (SDH; synonymous to mitochondrial complex II) in HPV, with particular emphasis on regional differences along the vascular bed and consequences for PAP and perfusion-to-ventilation matching, using mutant mice heterozygous for the SDHD subunit of complex II (SDHD+/−).

Methods and results
Western blots revealed reduced protein content of complex II subunits SDHA, SDHB, and SDHC in lungs of SDHD+/− mice, despite unaffected mRNA content as determined by real-time PCR. Hypoxic pulmonary vasoconstriction of small (20–50 μm) intra-acinar and larger (51–100 μm) pre-acinar arteries was evaluated by videomorphometric analysis of precision-cut lung slices. The hypoxic response was detectable in pre-acinar arteries but absent from intra-acinar arteries of SDHD+/− mice. In isolated perfused lungs, basal PAP and its hypoxia-induced increase were indistinguishable between both mouse strains. Arterial oxygenation was measured after provocation of regional ventilatory failure by tracheal fluid instillation in anaesthetized mice, and it declined more in SDHD+/− than in wild-type mice.

Conclusion
SDHD is required for the formation of a stable mitochondiral complex II and it is selectively important for HPV of intra-acinar vessels. This specialized vascular segment participates in perfusion-to-ventilation matching but does not significantly contribute to the acute hypoxic rise in PAP that results from more proximal vasoconstriction.

Keywords
Hypoxic pulmonary vasoconstriction ● Succinate dehydrogenase ● Complex II ● Intra-acinar artery

1. Introduction
Exposure of mammals to hypoxia induces constriction of pulmonary arteries to direct the blood flow from poorly oxygenated to well-oxygenated regions of the lung, thereby matching perfusion to ventilation.1,2 This response occurs rapidly and is unique to the pulmonary circulation, whereas hypoxia causes dilatation in systemic arteries.3,4 Notably, the reactivity and relative importance of individual sensor and effector pathways vary along the pulmonary vascular tree.

In the rat, rings of conduit pulmonary arteries respond to hypoxia with an initial small constriction followed by relaxation, whereas rings from vessels with external diameter below 300 μm react with a monophasic constriction. These differences are ascribed to differential expression of electrophysiologically distinct types of K+ channels.5 Neither the molecular nature of the oxygen sensor nor the effector pathway leading to hypoxic pulmonary vasoconstriction (HPV) is fully deciphered yet, although a number of proteins whose deficiency or blockade impairs HPV have been identified.
(reviewed in references 6–10). One of them is complex II of the mitochondrial respiratory chain, which is synonymous to succinate dehydrogenase (SDH). A hallmark feature of SDH is its dual involvement both in the respiratory chain and in the Krebs cycle.9 Succinate dehydrogenase consists of four protein subunits. SDHA is a flavoprotein catalysing the oxidation of succinate to fumarate in the Krebs cycle. The electrons originating from this oxidation are transferred to ubiquinone by the iron-sulphur protein SDHB. These two subunits are anchored to the inner mitochondrial membrane by the small hydrophobic subunits SDHC and SDHD, which are integral proteins of the inner mitochondrial membrane, bind haem and form cytochrome b.10,11 Evidence for the involvement of SDH in the oxygen sensing effector cascades derives from germline mutations in the mitochondrial complex II genes SDHB, SDHC, and SDHD, as well as in SDH5, which encodes a protein required for insertion of flavin adenine dinucleotide cofactor into SDHA. These mutations cause hereditary head and neck cancer,4 pulmonary hypertension syndrome in broilers,14 and using inhibitors that interfere either with substrate binding or with electron transfer from complex II towards ubiquinone, we have previously shown that SDH is required for hypoxic up-regulation of reactive oxygen species and for HPV of intrapulmonary arteries in the lung mouse.15,16

On this background we hypothesized that HPV is diminished in mice carrying a null allele of the SDHD gene. As homozygous (SDHD−/−) animals die at early embryonic stages, heterozygous (SDHD+/−) mice were investigated, because they show a generalized decreased level of complex II activity.17 In view of the differential characteristics of pulmonary arterial segments along the vascular bed,5,18 we set out to determine separately the hypoxic responses of small intra-acinar and larger pre-acinar arteries in murine precision-cut lung slices (PCLS), and of the pulmonary perfusion resistance in isolated, hypoxic ventilated lungs. The relevance of SDHD for perfusion-to-ventilation matching was evaluated by measuring arterial oxygenation after provocation of regional ventilatory failure. Collectively, our data show that mitochondrial complex II is selectively important for HPV in intra-acinar vessels, which translates into relevance for perfusion-to-ventilation matching but not for acute hypoxic pulmonary hypertension.

2. Methods

All experiments were approved by the Regierungspräsidium Giessen. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

2.1 Generation of SDHD+/− mice

The generation of 129SvJ mice heterozygous for the mitochondrial complex II subunit SDHD has been described earlier.17 All animals used in this study were from this colony with SDHD+/− (WT) mice serving as control animals.

2.2 DNA isolation and genotyping

Total DNA was isolated from tail cuts using DNasey Blood & Tissue Kit (Qiagen, Hilden, Germany). Subsequent PCR was performed using AmpliTaq Gold polymerase (Applied Biosystems, Darmstadt, Germany). The thermal cycling programme consisted of one cycle of 10 min at 95°C for denaturation, 40 cycles of 40 s at 95°C, 40 s at 62°C, 2 min at 72°C, and a final extension at 72°C for 7 min. In order to discriminate between the wild-type and the mutant allele, a three-primer approach was used (primers: 5′-TCAGTGACAACGTGAGCAC-3′, 5′-CAAGGT CCGAACCCAGAGAT-3′, and 5′-ATAGGCCAGCGTGGATGTCC-3′). The PCR products were separated by electrophoresis on a 1% Tris-acetate-EDTA gel.

2.3 RNA isolation and real-time RT-PCR

Total RNA was isolated from freshly prepared lung slices (n = 6) using RNeasy mini kit (Qiagen) according to the manufacturer’s protocol. To remove genomic DNA contamination, isolated RNA samples were treated with DNase (Invitrogen, Karlsruhe, Germany) for 15 min at 25°C. One microgram of RNA was used in a 20 μL RT reaction to synthesize cDNA using Superscript RNase H− Reverse Transcriptase (200 U/μg RNA; Invitrogen) and oligo dTs as primers. The RT reactions were performed for 50 min at 42°C. Real-time quantitative PCR was carried out using an I-cycler IQ detection system (Bio-Rad, Munich, Germany) in combination with the IQ SYBR Green Real-Time PCR Supermix (Bio-Rad). The thermal cycling programme consisted of initial denaturation in one cycle of 10 min at 95°C, followed by 40 cycles of 20 s at 95°C, 20 s at 59°C, and 20 s at 72°C. Primer sequences are provided in Table 1. The calculation of the gene expression was done as described previously.19 Sequencing of the PCR products was done by MWG Biotech (Ebersberg, Germany).

Table 1. Primers used for a quantitative analysis of the expression of mRNAs for complex II subunits SDHA, SDHB, SDHC, and SDHD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Product length</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHA</td>
<td>Forward</td>
<td>144 bp (1689−1833)</td>
<td>NM023281</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>140 bp (615−755)</td>
<td>BC051934</td>
</tr>
<tr>
<td>SDHB</td>
<td>Forward</td>
<td>117 bp (144−231)</td>
<td>NM025321</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>101 bp (166−267)</td>
<td>NM025848</td>
</tr>
<tr>
<td>SDHC</td>
<td>Forward</td>
<td>176 bp (154−340)</td>
<td>NM009735</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDHD</td>
<td>Forward</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2-MG</td>
<td>Forward</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Reverse</td>
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β2-Microglobulin (β2-MG) mRNA served as housekeeping gene control.
2.4 Western blotting and generation of antibodies against complex II subunits

Mice were killed by cervical dislocation and immediately exsanguinated by cutting the abdominal aorta. The isolated lungs were rapidly frozen in liquid nitrogen. Tissue homogenates were prepared in extraction buffer [7 M urea, 10% glycerol, 10 mM Tris–HCl pH 6.8, 1% sodium dodecyl sulphate, 5 mM dithiothreitol, 0.5 mM phenylmethylsulphonylfluoride, 1:10 concentrated Complete Mini Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany)] using a ball mill (Mixer Mill MM300; Retsch GmbH, Haan, Germany) for tissue desintegration. For western blotting, protein homogenates were resolved by 10% or 15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Schwabach, Germany). For detection of mitochondrial proteins, membranes were covered with affinity-purified polyclonal anti-SDHA (1:5000 dilution), anti-SDHB (1:20000 dilution), or anti-SDHC antibody (1:15 000 dilution), or with commercially available monoclonal antibody raised against the α-subunit of complex V (1:5000 dilution; Molecular Probes/Invitrogen, Karlsruhe, Germany). The three polyclonal antibodies for detection of complex II subunits were generated by immunization of rabbits with corresponding peptides (SDHA, aa581–596; SDHB, aa157–170; and SDHC, aa51–66; Pineda, Berlin, Germany) and affinity-purified by employing the corresponding peptide antigen. For detection of β-actin by the use of a monoclonal mouse anti-β-actin antibody (clone AC-15, 1:50 000 dilution; Sigma-Aldrich, Taufkirchen, Germany), one-tenth of the protein amount was applied to the gels. After washing the membranes with Tris-buffered saline (0.1% Tween 20, they were incubated with horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (both diluted 1:10 000 in Tris-buffered saline, 0.1% Tween 20, and 2.5% milk powder; Pierce, Rockford, IL, USA). Bound antibody was visualized by the enhanced chemiluminescent SuperSignal West Dura Extended Duration Substrate (Pierce). For a densitometric evaluation of the signals, X-ray films were scanned and mean intensities of immunoreactive bands calculated on a scale of grey values ranging from 0 to 255 using open-to-public software ‘ImageJ’ 1.37V.

2.5 Lung perfusion, ventilation, and hypoxic manoeuvres

Mouse lungs were perfused and ventilated as described previously.20,21 Briefly, animals were anaesthetized with ketamine (125 mg/kg bodyweight, i.p.) and xylazine (25 mg/kg bodyweight, i.p.) and anticoagulated with heparin (1000 U/kg) by intravenous injection. After intubation via a tracheostomy, mice were ventilated with room air (positive pressure ventilation) with a 250 μL tidal volume, 90 breaths/min, and 2 cmH2O positive end-expiratory pressure (Minivent Type 845; Hugo Sachs Elektro- nik, March-Hugstetten, Germany). Midsternal thoracotomy was followed by insertion of catheters into the pulmonary artery and left atrium. Using a peristaltic pump (ISM834A V2.10; Ismatec, Glattbrugg, Switzerland), buffer perfusion via the pulmonary artery was started at 4°C and set at 2.0 mmHg. Meanwhile, the flow was slowly increased from 0.2 to 2 mL/min, and the entire system was heated to 37°C. Pulmonary artery pressure (PAP) was registered via small-diameter catheters. After reaching a steady state, lungs were ventilated for 10 min with a hypoxic gas mixture consisting of 1% O2, 5.3% CO2, balanced with N2, followed by a 15 min period of normoxic ventilation (21% O2, 5.3% CO2, balanced with N2), after which a second hypoxic challenge of 10 min was performed. The increase in PAP (ΔPAP) was estimated during both hypoxic episodes. Normoxic PAP was assessed at the end of the steady-state period.

2.6 Measurement of arterial oxygenation after tracheal fluid instillation

Arterial oxygenation was analysed in mice after provocation of regional ventilatory failure. For this purpose, anaesthetized mice (125 mg ketamine/kg bodyweight and 25 mg xylazine/kg bodyweight, i.p.) were ventilated with room air and at time point zero challenged with an airway fluid load of 25 μL of saline by tracheal administration. Arterial oxygenation was measured by an ABL 500 (Radiometer, Copenhagen, Denmark) in blood drawn from the carotid artery directly before and 2, 5, 10, and 15 min after tracheal instillation.13 Anaesthesia was controlled by reflexes, including corneal reflex, pedal reflex, ear reflex, and muscle tone.

2.7 Preparation of murine PCLS and videomorphometric analysis

Precision-cut lung slices were prepared according to protocols described previously.15,16,22 At the beginning of each measurement, the viability of the transverse-sectioned vessel within the PCLS was checked by application of the thromboxane analogue 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F2α (U46619; 10 μM; Sigma Aldrich) to induce vasokonstriction. Subsequently, vessels were dilated by addition of the NO donor sodium nitroprusside (Nipruss; 30 μM; Schwarz Pharma GmbH Deutschland, Monheim, Germany). Next, the PCLS were exposed to normoxic or hypoxic gassed medium for 40 min, and at the end of the experiment viability of the arteries was again analysed by the addition of 10 μM U46619. The flow rates were 0.7 mL/min during incubation with normoxic (21% O2, 5% CO2, and 74% N2) or hypoxic medium (1% O2, 5% CO2, and 94% N2) and 6 mL/min for washing steps. The partial pressure of O2 of the media was 160 and 40 mmHg, respectively. Drug application was performed at flow arrest. The superfusion chamber was mounted on an inverted microscope (Leica, Wetzlar, Germany), and images of vessels were recorded by a CCD camera (Stemmer Imaging, Puchheim, Germany). Pictures were taken every 2 min using the Optimas 6.5 software (Stemmer Imaging). Changes in the vascular luminal area were evaluated as described previously.23 For graphic presentation of HPV, the value obtained immediately before exposure to normoxic or hypoxic gassed medium was set as 100%. The initial phases of the experiments in which the viability of the vessels were tested are not integrated in the graphs.

2.8 Statistical analysis

Statistical analysis was performed by using SPSS Base 15 or 18 (SPSS Software, Munich, Germany) and GraphPad InStat (Lajolla, CA, USA). Statistical significance between groups was determined with the Kruskal–Wallis test and, if P ≤ 0.05, subsequent Mann–Whitney U-test or ANOVA with the Student–Newman–Keuls post hoc test as appropriate; P ≤ 0.05 was considered significant and P ≤ 0.01 highly significant.

3. Results

3.1 Levels of SDHA, SDHB, and SDHC protein—but not mRNAs—are reduced in lungs of SDHD+/− mice

Genotyping of mice was performed by PCR analysis of genomic DNA from tail cuts, and heterozygous animals were identified by the appearance of a band of 1.25 kb in addition to the 1.8 kb product
detectable in WT mice (Figure 1). Quantitative RT-PCR of lung specimens revealed a reduction of SDHD mRNA by about 25% in SDHD+/− animals, whereas no differences were noted between SDHD+/− and WT mice in SDHA, SDHB, and SDHC mRNAs (Figure 2).

Western blots of lung extracts were run to analyse the expression of complex II subunits at protein level (Figure 3A−C). Quantification of the intensities of the immunoreactive bands disclosed a significant reduction of SDHA by 15%, of SDHB by 22%, and of SDHC by 32%. The polyclonal anti-SDHC antibody also reacted with a protein of 35 kDa, which was not detectable in extracts of purified mouse heart mitochondria (Figure 3C). This protein was present in comparable amounts in WT and heterozygous mice. Affinity-purified antisera raised against a synthetic peptide corresponding to amino acids 52–66 of SDHD proved not to be suitable for SDHD detection in western blots. The amount of the α subunit of complex V (ATP synthase), which is a component of the mitochondrial respiratory chain but not part of complex II, was unchanged in SDHD+/− mice compared with WT animals (Figure 3D). In addition, the amount of the non-mitochondrial protein β-actin did not differ between samples from both mouse strains (see Supplementary material online, Figure S1), demonstrating a selective reduction of complex II subunits in SDHD+/− mice.

### 3.2 The acute hypoxia-induced increase in PAP is unimpaired in SDHD+/− mice

The acute hypoxic pressure response of the pulmonary vascular system was investigated in isolated, buffer-perfused and ventilated mouse lungs. Wild-type and heterozygous mice exhibited comparable basic PAP (Figure 4A). Two successive periods of hypoxic ventilation resulted in distinct increases in PAP. The extent of pressure rise did not differ between SDHD+/− and WT mice (Figure 4B).

### 3.3 HPV of small intra-acinar arteries but not of pre-acinar arteries is lost in SDHD+/− mice

Videomorphometry of PCLS allows the individual examination of HPV of separate size classes of intra-pulmonary vessels. First, we analysed small intra-acinar arteries with inner diameters between 20 and 50 μm, which lack an adventitial layer and are directly attached to the alveolar septa.16 In WT mice, HPV of these vessels manifested as a 30% reduction of vascular luminal area upon perfusion of the PCLS with hypoxic gassed medium (Figure 5A). In PCLS of SDHD+/− mice, however, no hypoxia-induced changes in luminal diameter of vessels of this size class were observed (Figure 5A). However, these vessels retained full contractile responsiveness to U46619, which was applied cumulatively in a concentration range of 0.1 nM to 10 μM (Figure 5B), demonstrating the selectiveness of loss of HPV in small intra-acinar arteries of SDHD+/− mice.

Next we analysed the hypoxic response of pre-acinar arteries with an inner diameter between 51 and 100 μm (Figure 5C). These vessels are present along the airways, possess a complete muscle layer, and are surrounded by adventitial loose connective tissue. In contrast to small intra-acinar vessels, pre-acinar arteries of both SDHD+/− and WT mice reacted with a significant reduction in luminal area when exposed to hypoxic gassed medium. There was a slight trend towards a smaller extent of this HPV in SDHD+/− compared with WT mice, but differences were not significant.

### 3.4 Arterial oxygenation after provocation of regional ventilatory failure is worse in SDHD+/− mice

To test whether the observed lack of intra-acinar HPV translates into impairment of oxygen-regulated perfusion-to-ventilation matching, we measured the arterial oxygenation in anaesthetized mice after provocation of regional ventilatory failure by tracheal administration of 25 μL saline (Figure 6). In both mouse strains, the arterial partial
pressure of O₂ dropped within 2 min after this manoeuvre and stayed low until the end of the observation period (15 min after instillation). In the early phase (2 and 5 min) after fluid instillation, the decline in arterial partial pressure of O₂ was significantly more severe in SDHD+/− than in WT mice (Figure 6).  

4. Discussion

This study shows that SDHD is selectively important for HPV of intra-acinar vessels, which translates into relevance for perfusion-to-ventilation matching but not for the acute hypoxic rise in pulmonary perfusion resistance. Loss of one allele for SDHD resulted in a reduction in the amount of protein of subunits SDHA, SDHB, and SDHC in the lung. This is in line with data demonstrating an approximately 50% lower SDH activity in mitochondria isolated from various organs of SDHD+/− mice compared with WT animals and with distinctly reduced amounts of complex II subunits in heart homogenates of SDHD+/− mice.17,24 Likewise, SDHD mutations in head and neck paragangliomas result in destabilization of complex II, reduced SDHB content, and loss of enzymatic activity.15–27 SDHD, together with SDHC, serves as membrane anchor for the catalytic subunit SDHA and the electron transferring subunit SDHB.10 Presumably, the diminished expression of pressure of O₂ dropped within 2 min after this manoeuvre and stayed low until the end of the observation period (15 min after instillation). In the early phase (2 and 5 min) after fluid instillation, the decline in arterial partial pressure of O₂ was significantly more severe in SDHD+/− than in WT mice (Figure 6).  

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Complex II is essential for HPV of intra-acinar arteries
SDHD impairs assembly of complex II, leading to accelerated degradation of the subunits, as evidenced also from cell culture studies.\(^{29}\) On this background, the impairment of intra-acinar HPV observed in the present study in SDHD\(^{+/−}\) mice cannot be ascribed specifically to SDHD subunit deficiency but rather to general complex II disturbance.

Various intracellular pathways have been described that link complex II to oxygen-regulated mechanisms. Dysfunctional SDH results in accumulation of its substrate, succinate,\(^{29}\) which is an inhibitor of hypoxia-inducible factor (HIF) prolyl hydroxylases.\(^{30,31}\) Thereby, reduced SDH activity is linked to reduced HIF hydroxylatation, increased HIF stabilization, and thereby enhanced HIF-driven gene transcription. This pathway is considered to be relevant for tumourigenesis in patients with SDHB, SDHC, and SDHD mutations,\(^{1,12,32}\) but may not account for rapid events such as HPV, which develops within minutes. Succinate is also a ligand of the G-protein-coupled receptor GPR91, which has a major role in retinal angiogenesis\(^{33}\) and is expressed in the lung as well.\(^{34}\) We previously demonstrated that succinate blocks HPV in murine intra-acinar arteries,\(^{16}\) so that succinate accumulation represents one possible mechanism of linking diminished SDH activity to abrogation of intra-acinar HPV. On the other hand, complex II is also a site of generation of reactive oxygen species, both during hypoxic conditions and when mutations lead to expression of dysfunctional subunits.\(^{35,36}\) This mechanism has also been linked to HIF stabilization and tumourigenesis,\(^{36}\) as well as to intra-acinar HPV.\(^{15,16}\) The present study was not aimed to clarify which of these, or currently unknown other, mechanisms translates complex II impairment in SDHD\(^{+/−}\) mice into loss of HPV, but rather focused upon the regional differences of complex II involvement in HPV along the pulmonary vascular tree and the resulting consequences for pulmonary perfusion resistance and blood oxygenation.

Notably, loss of one allele of the SDHD gene abrogated the hypoxic response of small (20–50 \(\mu\)m) intra-acinar pulmonary arteries, but not that of larger pre-acinar arteries. Likewise, it does not block hypoxic responsiveness of the carotid body, the main arterial chemoreceptor that senses oxygen levels in the blood, although electrophysiological properties (\(K^+\) currents) of carotid body glomus cells are affected in SDHD\(^{+/−}\) mice, resulting in an increase in the tonic secretory activity.\(^{37}\) This supports the hypothesis that SDH deficiencies may affect the hypoxic response of certain tissues by altering the effector pathways. Regional diversity of the pulmonary arterial system along its progression from the right heart to the pulmonary capillaries has long been recognized and includes sensitivity to hormones and neurotransmitters as well as its reactivity to hypoxia. In the rabbit, the contractile profile to KCl, phenylephrine, adrenaline, histamine, serotonin, and prostaglandin \(F_{2\alpha}\) differs markedly between large extrapulmonary, large intralobular, and small intralobular arteries.\(^{37}\) 5,6-Epoxycosatrienoic acid relaxes extralobar but constricts intralobar rat pulmonary arteries.\(^{38}\) In the rat, conduit pulmonary artery rings respond to hypoxia with an initial small constriction followed by a relaxation below baseline, whereas rings from vessels with external diameter below 300 \(\mu\)m react with a monophasic constriction.\(^{3,39}\) Kübler and co-workers have established an elegant system of intravital microscopy, which allows the study of the pulmonary microcirculation at the surface of the lung of anaesthetized mice. They observed a marked hypoxic response in medium-sized arterioles (diameter 30–50 \(\mu\)m), but only weak HPV in small arterioles (diameter 20–30 \(\mu\)m).\(^{40}\) The 7–8% hypoxia-induced decrease in diameter of medium-sized arterioles fits well to our data for intra-acinar vessels, for which we determined a 20% decrease of the luminal area, which corresponds to a 10% reduction in diameter. This demonstrates that our videomorphometric analysis of PCLS gives a good reflection of the situation in vivo.

The present study is the first to identify a genetic model which causes selective loss of HPV in one particular segment of the pulmonary arterial bed. Besides shedding light upon the role of SDH in HPV, this model allows more general conclusions to be drawn on the specific role of the intra-acinar pulmonary artery. This specialized segment of the pulmonary arterial tree does not contribute significantly to the overall pulmonary vascular perfusion resistance, because PAP rose normally in isolated hypoxic perfused lungs from SDHD\(^{+/−}\) mice, despite the lack of constriction of intra-acinar arteries in these conditions. Accordingly, the hypoxic increase of PAP in the

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**Figure 5** Videomorphometric analysis of the hypoxic response of intra-acinar arteries in precision-cut lung slices (PCLS; A). Vasoreactivity was recorded as relative changes of the luminal area of cross-sectioned vessels against time. Intra-acinar arteries of WT mice responded to incubation with hypoxic-gassed medium with a distinct contraction, which was not observed in intra-acinar arteries of SDHD\(^{+/−}\) mice (A). The response of intra-acinar arteries to increasing concentrations of U46619 was comparable in both mouse strains (B). Pre-acinar arteries of both WT and SDHD\(^{+/−}\) mice contracted in response to exposure to hypoxia (C). The difference in the extent of HPV between both mouse strains did not reach significance. Data are presented as means ± SEM. ‘\(n\)’ in parentheses refers to the number of arteries/number of animals from which PCLS were made. Tests of significance were done at the time points indicated by arrows. *\(\#\) WT normoxia (Norm) was tested against WT hypoxia (Hyp); \(\#\) SDHD\(^{+/−}\) Norm was tested against SDHD\(^{+/−}\) Hyp; \(\#\) WT Hyp was tested against SDHD\(^{+/−}\) Hyp. **\(P≤0.01\), \#\(P≤0.05\), \#\#\(P≤0.01\), §§\(P≤0.01\) (Mann–Whitney U-test).

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**Figure 6** Arterial oxygenation in anaesthetized, ventilated mice after tracheal administration of 25 \(\mu\)L saline. Two and five minutes after fluid instillation, arterial oxygenation was significantly lower in SDHD\(^{+/−}\) than in WT mice. *\(P≤0.05\) (ANOVA with post hoc test).
mouse lung is primarily determined by pre-acinar arteries. This conclusion is supported by the peculiar structure of small intra-acinar arteries with a discontinuous smooth muscle layer and the presence of contractile cells differing morphologically from those of larger pulmonary arterial branches. \(^{16,41,42}\) Still, we observed a marked HPV of this vascular segment in WT mice, consistent with studies on rat pleural vessels by laser scanning luminescence microscopy. \(^{39}\) intravital microscopy of the murine pulmonary microcirculation, \(^{40}\) and our preceding videomorphometric studies. \(^{16}\) This mechanism contributes to perfusion-to-ventilation matching, because the acute decline in arterial oxygenation upon intratracheal fluid instillation was more severe in SDHD \(^{1/2}+\) than in WT mice.

In conclusion, loss of one allele of the SDHD gene is associated with reduced protein content of the other SDH subunits and results in selective loss of HPV at the level of the weakly muscularized intra-acinar artery, whereas larger pre-acinar arteries retain the capability for HPV. Together with PAP recordings from isolated perfused lungs and analysis of arterial oxygenation in animals challenged by intratracheal fluid instillation, these data suggest an involvement of intra-acinar arteries in directing blood to well-ventilated areas at the acinar level without contributing significantly to overall pulmonary arterial pressure, which is determined more proximally. While a previous study on TRPC6 gene-deficient mice revealed concomitant impairment of the hypoxic rise in PAP and arterial oxygenation, \(^{22}\) the present genetic model demonstrates that these parameters can, in principle, be influenced differently.

### Supplementary material

Supplementary material is available at Cardiovascular Research online.

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