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REVIEW

Hypoxic Pulmonary Vasoconstriction: An Important Component of the Homeostatic Oxygen Sensing System

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Summary

Hypoxic pulmonary vasoconstriction (HPV) rapidly and reversibly matches lung ventilation (V) and perfusion (Q), optimizing oxygen uptake and systemic oxygen delivery. HPV occurs in small pulmonary arteries (PA), which uniquely constrict to hypoxia. Although HPV is modulated by the endothelium the core mechanism of HPV resides in PA smooth muscle cells (PASMC). The PASMC's mitochondrial oxygen sensor lies within the electron transport chain (ETC) and includes NDUFS2 in ETC Complex-I. PASMC mitochondria respond to hypoxia by varying production of reactive oxygen species (ROS) and hydrogen peroxide in proportion to alveolar oxygen tension. Hypoxic ROS inhibition results in a state of reduction which triggers a redox-mediated inhibition of oxygen-sensitive, voltage-gated, potassium channels, including Kv1.5 and Kv2.1. Kv channel inhibition depolarizes the PASMC, opening of large-conductance calcium channels (Ca_L), elevating cytosolic calcium and activating the contractile apparatus. HPV is strongest in small PAs where sensors (hypoxia-responsive mitochondria) and effectors (oxygen-sensitive K^+ channels) are enriched. Oxygenation at birth reverses fetal HPV, contributing to the rapid neonatal drop in pulmonary vascular resistance (PVR). A similar mitochondrial-K^+ channel sensor-effector mechanism exists in the ductus arteriosus (DA), however in DASMC it is oxygen-induced increases in mitochondrial ROS that inhibit DASMC K^+ channels, causing DA constriction. Atelectasis and pneumonia elicit HPV, which optimises V/Q matching, increasing systemic oxygenation. Whilst HPV in response to localized hypoxia in a single lung lobe does not increase PA pressure; global airway hypoxia, as occurs with altitude or sleep apnea, causes pulmonary hypertension. HPV can be inhibited by drugs, including calcium channel blockers, or used to maintain a dry operative field during single lung anesthesia for lung surgery. HPV does not normally cause lung edema but excessive, heterogenous HPV contributes to high altitude pulmonary edema. HPV is suppressed in COVID-19 pneumonia by a SARS-CoV-2 mitochondriopathy. HPV is a component of the body's homeostatic oxygen sensing system.

Keywords

Ductus arteriosus • Redox • NDUFS2 • Oxygen sensitive potassium • Channels • High altitude pulmonary edema (HAPE) • Mitochondrial electron transport chain • COVID-19 pneumonia • Atelectasis

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Introduction

Hypoxic pulmonary vasoconstriction (HPV) was reported first in 1894 by Bradford and Dean [1], and subsequently more precisely described by von Euler and Liljestrand [2]. In the Bradford and Dean paper hypoxic stimulation was achieved by transient asphyxia (a form of global airway hypoxia), whereas in the more definitive work of von Euler and Liljestrand the lung was ventilated with hypoxic gas mixtures (Fig. 1). These authors

concluded *"The experiments seem to warrant the conclusion, that the regulation of the pulmonary blood flow is mainly mediated by a local action of the blood and alveolar gases leading to an adequate distribution of the blood through the various parts of the lungs according to the effeciency [sic] of aeration.*" [2].

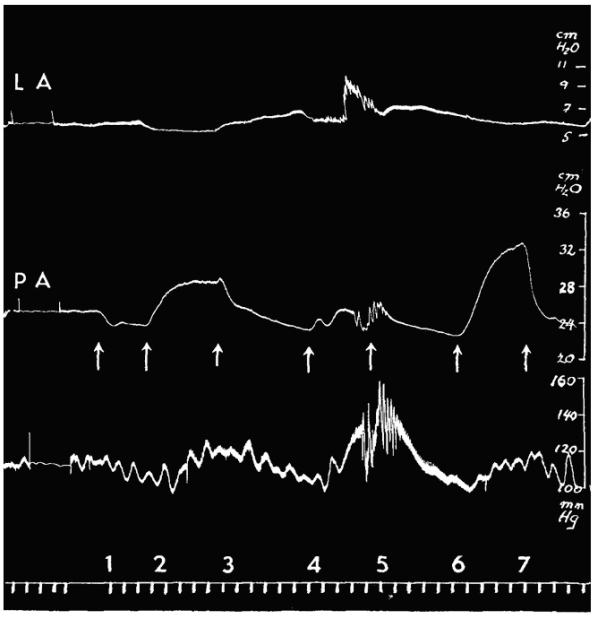

Fig. 1. Original description of HPV by von Euler and Liljestrand: Experiments were performed in a cat under chloralose anesthesia. The uppermost trace shows pressure in left auricle, the middle trace pulmonary arterial pressure, and the lower trace shows systemic blood pressure. The cat received artificial respiration and the thorax was open. Time scale is in units of 30 seconds.
Note point 6, which reflects hypoxic ventilation. HPV is rapid in onset, fully reversible, and not associated with significant changes in systemic blood pressure.
1. Oxygen (room air)
2. 6.5 % CO_2 in oxygen
3. Oxygen (room air)
4. 18.7 % CO_2 in room air
5. Oxygen (room air)
6. 10.5 % oxygen in nitrogen (hypoxia)
7. Oxygen (room air).
Reproduced with permission from [2].

HPV has long intrigued physicians and physiologists perhaps because it is involved in so many physiologic and pathologic events in life, including the transition of the fetal circulation at birth (withdrawal of HPV), the regulation of ventilation/perfusion matching to optimize systemic oxygenation in normal lungs, counteracting intrapulmonary shunting of blood in pneumonia and atelectasis, and mediating the pulmonary hypertensive response to ascent to altitude. When excessive and heterogenous, HPV also contributes to high altitude pulmonary edema (HAPE). Loss of HPV, whether caused by drugs that are pulmonary vasodilators or due to infectious agents, as occurs with COVID-19 and coronavirus infection [3], can exacerbate systemic hypoxia by causing V/Q mismatch and intrapulmonary shunting (perfusion of unventilated lung segments).

At the heart of HPV is the unique ability of small pulmonary arteries (PAs) (<400 μm diameter) to constrict to hypoxia. Virtually all systemic arteries (and large PAs) relax upon exposure to hypoxia [4-6]. We have made the case that intrapulmonary arteries (PA)s are part of the body's homeostatic oxygen sensing system (HOSS), a system which includes the carotid body, ductus arteriosus, and fetal placental arteries [7]. These specialized HOSS tissues each initiate changes in vascular tone and/or neural output that serve to optimize systemic oxygen delivery. For example, at altitude the type 1 cells within the carotid body trigger hyperventilation to increase oxygen uptake from rarefied air. Likewise, at birth the ductus arteriosus SMC constrict, achieving functional DA closure which diverts blood from the right ventricle (RV) into the pulmonary circulation of the new ventilated lung. Simultaneously, with the first breath, the neonatal PASMC relax as the lung's fetal hypoxic milieu is replaced with the oxygen-rich neonatal environment. The withdrawal of HPV relaxes the pulmonary circulation, preparing it to receive the RV output at low PA pressure. One could argue that systemic arteries, by dilating to hypoxia, also contribute to optimizing systemic oxygen delivery in states of hypoxia and thus may too be considered part of the HOSS. The tissues of the HOSS (at least the type 1 cell carotid body, DASMC, PASMC) all use mitochondria as oxygen sensors and ion channels, notably potassium and calcium channels, as effector mechanisms [7].

It should be noted that the HOSS, as originally described [7], uses conserved mitochondrial-ROS-ion channel signalling pathways in specialized tissues to rapidly (in seconds) elicit adaptive responses to counteract hypoxia. However, there are equally important response to hypoxia that occur more slowly in response to hypoxia in most tissues. These transcriptional responses are often initiated by redox triggers (including ROS) but are mediated by transcriptional regulators, such as hypoxia inducible factors like HIF-1α [8] and prolyl hydroxylases. These transcriptional hypoxic responses onset more slowly and are sustained throughout exposure to hypoxia. The HIF-1α pathways interact with the mechanism of HPV. For example, patrial deficiency of

HIF-1α in mice impairs acute HPV (specifically impairing hypoxia-induced depolarization and K^+ current inhibition in PASMC from HIF-1α heterozygous knockout mice) and inhibits chronic hypoxic pulmonary hypertension [9]. Moreover, the acute mitochondrial oxygen sensing pathway is involved in regulation of HIF-1α. For example, in pulmonary arterial hypertension there is normoxic activation of HIF-1α due to epigenetic silencing of mitochondrial superoxide dismutase 2, which reduces production of mitochondrial-derived hydrogen peroxide [10]. Normoxic activation of HIF-1α, whether spontaneous or achieved by cobalt exposure, rapidly activates dynamin related protein 1 (Drp1) in human PASMC which triggers mitotic mitochondrial fission, changes mitochondrial redox control [11] and promotes the hyperproliferation of PASMC. Thus, there is interaction between acute mitochondrial-redox mechanisms of oxygen sensing in the HOSS and longer-term transcriptional responses to hypoxia both in HOSS tissues and less specialized tissues.

HPV has certain hallmarks which any proposed mechanism must address. HPV is rapid in onset, beginning within seconds of exposure to even mild airway hypoxia [12,13]. Moreover, although HPV can be sustained for pronged periods [14], it is rapidly reversible when airway hypoxia is relieved. HPV is also dependent, in large part, on influx of extracellular calcium [15]. However, there is also evidence for a role for hypoxia-induced release of intracellular calcium and activation of rho kinase in triggering HPV, see review [16]. Although many endothelium-derived vasodilators (notably nitric oxide (NO), prostaglandins) and vasoconstrictors (notably endothelin) attenuate and enhance HPV, respectively, HPV can be elicited in PA rings denuded of endothelium or even in isolated PASMC [17]. Nonetheless, the magnitude of HPV is clearly augmented by inhibition of nitric oxide synthase [18,19] or cyclooxygenase [20].

The cellular electrophysiology of HPV

In 1992 a partnership between the Weir-Archer lab and the laboratory of Professor Joe Hume made the first demonstration that hypoxia inhibited the outward K^+ currents of canine PASMC, leading to membrane depolarization. This hypoxic inhibition of I_K was unique to PASMC and did not occur in canine renal arterial SMCs [21]. In PASMC voltage-gated potassium channels (Kv) maintain a resting membrane potential of ~ -60 mV. The membrane potential is set by a tonic efflux of positively charged potassium ions down a concentration gradient of ~ 140 mM (intracellular) to 5 mM (extracellular). The resulting negative membrane potential decreases the open state probability of voltage-gated, L-type calcium channels (Ca_L). With hypoxic PASMC depolarization Ca_L have more and longer openings and extracellular Ca^{2+} enters the PASMC, traveling down a 20,000:1 extracellular:intracellular Ca^{2+} gradient, reviewed in [22]. The resulting increase in cytosolic calcium triggers vasoconstriction, which is subsequently reinforced by Rho-kinase-mediated phosphorylation of myosin light chain, which mediates calcium sensitization contributing to a sustained phase of pulmonary arterial vasoconstriction [15,21]. Based on this cellular electrophysiology, performed using the whole-cell patch clamp technique, it is not surprising that the pulmonary circulation in isolated perfused rat lungs constrict in response to the Kv channel inhibitor, 4-aminopyridine [23]. Moreover, HPV is inhibited by Ca_L inhibitors, like nifedipine and verapamil, while it is enhanced by CaL agonists like BAYK8644 [15,24].

Molecular electrophysiology of HPV

After identification of the role of K^+ channels in HPV a search began to obtain molecular precision in specifying which of the many candidate Kv channels (or other channels, like TASK channels) was involved. Ultimately a key role for Shaker channels, notably Kv1.5, and Kv2.1 [25,26], was identified. Subsequently we showed that a Kv1.5 knockout mouse had reduced HPV [27]. However, there is evidence for involvement of other classes of K^+ and Ca^{2+} channels in the mechanism of HPV [28], including two-pore acid sensitive K^+ channels (TASK) [29,30]. TASK-1 channels are active at very negative membrane potentials and contribute to regulation of the resting membrane potential in PASMC and may also modulate the sensitivity of membrane potential to acid-base disturbances [31]. However, TASK-1 is not essential for HPV in murine PAs, since HPV persists in TASK-1 knockout mice and in the presence of a chemical TASK-1 inhibitor (A293) [32]. Nonetheless, mutations in the gene encoding TASK, *KCNK3*, predispose to WHO Group 1 pulmonary hypertension (pulmonary arterial hypertension, PAH) in transgenic rats [33] and humans [34].

The mitochondrial electron transport chain (ETC) is an O_2-sensor in DA and adult PA SMC [35,36].

One of the earliest papers to identify a potential role for the mitochondrial ETC in HPV was by Rounds and McMurtry [37]. They found that five substances that blocked different steps of oxidative ATP production (10 mM azide, 1 mM cyanide, 1 mM dinitrophenol, 5 or 10 µM antimycin A, or 0.5 µM rotenone) trigger pulmonary vasoconstriction, which like HPV, was inhibited by the calcium channel blocker, verapamil [37]. Subsequently we showed rotenone and antimycin did indeed mimic hypoxia but do not achieve this by causing bioenergetic depletion, rather they inhibit normoxic ROS production, thereby regulating redox-sensitive potassium channels in a manner mimicking authentic hypoxia [38,39].

The ETC conducts electrons from donors derived from Krebs' cycle (NADH and $FADH_2$), down an electrochemical gradient to molecular O_2 (Fig. 2). These donors transfer electrons to Fe-S clusters, within the quinone chambers (Q-site) of Complex I and III. One such Fe-S center, NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 (NDUFS2) in Complex I, is a source of oxygen-sensitive, mitochondrial-derived ROS (Fig. 2) and is implicated in O_2-sensing in adult PASMC and carotid body type-1 cells [17,40]. During electron transfer ~1-3 % of electrons leak from Q-sites, such as NDUFS2 in Complex I and the Rieske Fe-S center in Complex III [41,42]. More contemporary measurements of electron leakage from the ETC suggest that electron leak comprises < 0.5 % of total electron flux under normal conditions [43-45].

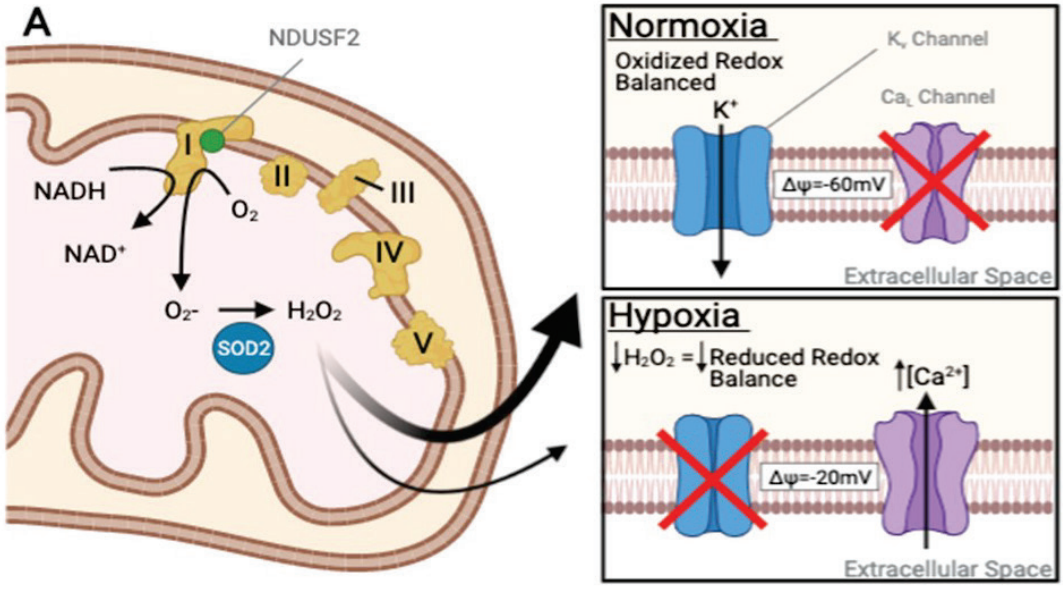

Fig. 2. Mitochondrial O_2-Sensing in PASMC: In adult, normoxic, PASMC mitochondria generate superoxide from NDUFS2 in ETC Complex I. This is dismutated to hydrogen peroxide by superoxide dismutase 2 (SOD2). Hydrogen peroxide diffuses to plasmalemmal Kv channels which it oxidizes and opens. Inset on **right**: In normoxia, the resulting hyperpolarization of the SMC membrane potential decreases the opening of large conductance, voltage-gated calcium channels (Ca_L), lowering $[Ca^{2+}]_i$ and relaxing tone. In hypoxia the pathway reverses, causing HPV. It is unknown if this pathway is conserved in fetal PASMC.

Uncoupled electron leak generates superoxide anion ($O_2^{\bullet-}$). $O_2^{\bullet-}$ is rapidly changed by mitochondrial superoxide dismutase 2 (SOD2) into H_2O_2, which serves as a diffusible, redox signaling molecule, connecting ETC function to cellular electrophysiology (Fig. 2). Mitochondrial ROS control vascular tone by regulating the gating kinetics of redox-sensitive ion channels, such as the voltage-gated potassium channel, Kv1.5, and enzymes, such as rho kinase [25,36,46-49]. In adult PA and fetal DA, inhibitors of ETC Complex I (rotenone) or Complex III (antimycin A) mimic hypoxia's opposing effects on ROS production and vascular tone [36,39,41,50-52]. Like hypoxia, inhibitors of ETC Complex I (rotenone) and -III (antimycin A) inhibit K^+ currents and cause PA constriction [39]. The parallel effects of hypoxia and ETC inhibitors on subcellular signaling (K+ channel opening in DASMC versus inhibition in PASMC) and vascular tone (DA vasodilatation versus PA vasoconstriction) suggest a role for mitochondrial *spatial heterogeneity* in

the opposing PA and DA O_2-responses. The effects of hypoxia and ETC inhibitors are mimicked by reducing agents (e.g., duroquinone). Conversely, oxidants (e.g., diamide) mimic oxygen and increase I_K while causing pulmonary vasodilatation [46].

Molecular Identification of the O_2 sensor in HPV: Identification of NDUFS2 as a mitochondrial O_2 sensor in adult PASMC

In 2019 we used a targeted proteomic strategy in mitochondria derived from adult rat PAs vs renal arteries (RAs), which dilate to hypoxia, to show that NDUFS2 is essential for acute O_2-sensing [17]. We isolated mitochondria from PA vs RA SMC and performed ETC complex pull-down by immunocapture to resolve the 45 ETC Complex I proteins. NDUFS2 expression was greater in PASMC vs RASMC. Small inhibitory RNA targeting NDUFS2 (siNDUFS2), selectively inhibited normoxic ROS production and suppressed HPV without altering responses to other vasoconstrictors or the function of RASMC. We also showed that isolated mitochondria derived from normoxic lungs were producing hydrogen peroxide during normoxia and the media surrounding these mitochondria inhibited HPV, when infused into an isolated lung bioassay model. We also found that redox-sensitive Ndufs2 cysteine residues within the lung were reduced during hypoxia and showed that both hypoxia and reducing agents caused functional inhibition of ETC-I. Thus, we concluded that NDUFS2 is an O_2-sensor in ETC Complex I of adult PASMC and regulates the downstream effector mechanism by generating ROS which are converted to hydrogen peroxide [17]. Although mitochondria were not initially believed to be oxygen sensors in the carotid body, this position has been revised. López-Barneo *et al.* in 2015 demonstrated that knockdown of a component of ETC Complex I, Ndufs2, selectively eliminated O_2 sensing in the mouse carotid body [40], much as occurs in rats in which Ndufs2 in the lung is depleted *in vivo* by siRNA, in which HPV is selectively inhibited [17].

Regional heterogeneity in the expression of O_2 sensitive K+ channels

The pulmonary circulation displays heterogeneity in the distribution of ion channels along its length. The proximal PASMC (derived from conduit arteries that display no vasoconstriction or weak vasoconstriction in response to hypoxia) are enriched in large conductance, calcium-sensitive, K^+ channels (BK_{Ca}) whereas the resistance PASMC, the site of HPV, are enriched in Kv channels. Moreover, Kv1.5 and Kv2.1, two key O_2-sensitive channels, account for much of the O_2-sensitive current in resistance-level PASMC [25]. HPV occurs in this Kv-enriched resistance PA portion of the pulmonary vasculature, in part, because resistance PASMCs preferentially express these O_2-sensitive Kv-channels [53]. This work was subsequently confirmed and extended by the group of Dr. Jason Yuan, who also noted the electrophysiologic heterogeneity of PASMC, which they showed partially relates not only to differential expression and function of Kv channels but also to regional differences in intrinsic mechanisms involved in regulating cytosolic calcium [54].

Mitochondrial diversity in pulmonary versus systemic arteries

We have studied the role of mitochondrial heterogeneity in the opposing O_2-responses in adult PA versus systemic arteries. The composition of mitochondrial ETC megacomplexes I and III differs between adult PA (which constrict in hypoxia) and adult renal arteries (RA), which relax in hypoxia) [5]. In renal artery SMC (RASMC), hypoxia increases mitochondrial ROS and Kv current, leading to RA relaxation; whilst the opposite occurs in PAs [5]. Complex I inhibitors also elicit opposing ROS responses and hemodynamic changes in PA vs RA (constriction vs relaxation) [5], illustrating that acute changes in ETC complex function can generate opposing ROS signals and vasomotor responses in adult arteries. Compared to RASMC, adult PASMC mitochondria also have lower O_2-consumption rates (OCR), a more depolarized mitochondrial membrane potential (ΔYm), and higher rates of normoxic mitochondrial ROS production. These functional differences in mitochondria are associated with lower expression of several ETC Complex I and Complex III subunits and higher expression of the H_2O_2-generating enzyme, mitochondrial SOD2 [5] in PASMC vs. RASMC. This spatial ETC heterogeneity confers upon adult PASMC a unique ability to vary ROS in proportion to PO_2, which is key to O_2-sensing. We propose that heterogeneity in ETC Complex structure and function underlies opposing O_2-sensing in adult PA vs RA [5].

Controversies in the mechanism of HPV

The role of mitochondria as oxygen-sensors is generally accepted, as is the hypothesis that ETC-derived ROS regulate the downstream ion channel effector

pathway (the gating of K^+ and Ca_L channels) that elicit vasoconstriction [38,39,55]. This fits nicely with the original Redox hypothesis of HPV published by Archer, Will, and Weir in 1986 [56]. However, disagreement persists as to whether, in adult PAs, physiologic levels of hypoxia (anoxia is not a stimulus for HPV) increase or decrease ROS/H_2O_2 levels [28,57-61]. Some groups, notably the Schumacker group at Northwestern University, Chicago, have shown that hypoxia increases mitochondrial ROS production, which they attribute to an hypoxic inhibition of the distal ETC, particularly at ETC Complex III [59,60]. They propose that inhibition of the distal ETC by hypoxia causes a net reduction of sites within the proximal ETC. The addition of electrons due to distal ETC inhibition and retrograde electron flux serves to increase electron leak from the proximal ETC and react with O_2 to form ROS. In contrast, our group, consistent with findings by the Wolin group [55,62-64], finds that PASMC ROS production increases in direct proportion to PO_2 and decreases with hypoxia. Production of ROS (including the major signalling molecule, hydrogen peroxide) falls in hypoxia due a reduced rate of electron flux (and a related decrease in electron leak) caused by decreased availability of molecular oxygen, the ETC's terminal electron acceptor [7]. It appears the hypoxic fall in ROS is associated with a reduction in oxygen consumption rate (OCR). We recently showed for example that siNDUFS2, which eliminates HPV and reduces ROS, reduces OCR in PASMC [17]. We also believe that reduced availability of O_2 as an acceptor of the leaked electrons may contribute to reduced ROS in hypoxia, although our data suggest this reflects inhibition of antegrade electron flow, rather than enhanced retrograde electron flow.

This direct relationship between PO_2 and ROS production is evident in other tissues in the HOSS, such as the ductus arteriosus (DA) in which ROS in DASMC increase with rising PO_2 at birth [52,65]. A direct relationship of ROS and PO_2 is also seen in non-HOSS tissues, like the heart. When the heart is studied in a Langendorff model and ROS are measured from the cardiac surface using enhanced chemiluminescence, ROS levels fall from a normoxic baseline during global cardiac ischemia and then increase far above baseline with reoxygenation, accounting for the reperfusion phase of myocardial ischemia-reperfusion injury [66]. This oxygen-induced rise in ROS in cardiac ischemia-reperfusion injury is largely due to mitochondrial fission mediated by dynamin related protein 1 (Drp1) in cardiac myocytes [67]. Although we find a direct relationship between PO_2 and ROS production, Lopez-Barneo's laboratory found the opposite relationship in type 1 cells of the carotid body in a study where they identified the role of Ndufs2 in response to hypoxia in these cells [40].

The basis for the discrepancies between those that find hypoxia to be an oxidized state with high ROS [59,60] versus those that report hypoxia is a reduced state with low ROS [7,61] remains unclear. However, we have identified and reported several methodological discrepancies amongst groups related to the use (or lack of use) of: (i) freshly isolated PASMC from resistance arteries and isolated perfused lungs (ii) focus on physiologic hypoxia (versus anoxia), (iii) attention to/reporting of pH and PCO_2, and (iv) performing dynamic and accurate ROS measurement in subcellular compartments [68]. The proposal that hypoxia is a chemical state of reduction (not oxidation), is supported by the observations that the opposing effects of hypoxia on PA versus systemic vascular tone (constriction versus dilation) and the opposing effects of hypoxia on cellular electrophysiology in PASMC versus systemic arterial SMC are mimicked by reducing agents (such as dithiothreitol), and not reproduced by exogenous administration of ROS and oxidants [5,50]. Any proposed mechanism of HPV should be judged by the degree to which it accounts for the physiological core properties of HPV, notably the response onset should be rapid (as HPV onsets within a few breaths of hypoxia) [13], reversible (like HPV), and should not induce edema [68]. A unifying theory for oxygen sensing should also (in our view) *"account for the opposing effects of hypoxia on tone in the pulmonary circulation (constriction) versus the ductus arteriosus and systemic vasculature (vasodilatation)"* [68].

We acknowledge that the use of ETC inhibitors like rotenone and antimycin A to study redox signaling is suboptimal as the effects of these agents may be confounded by the possibility that they inhibit mitochondrial metabolism. Therefore, Brand *et al.* developed electron leak suppressors [43], S1QEL and S3QEL. S1QEL prevents electron leak and superoxide production (both in the forward and reverse direction of electron flux) from site I_Q in ETC Complex 1 [45]. We recently used S1QEL to study oxygen sensing in the DASMC and showed that S1QEL, but not S3QEL, reversed oxygen-induced constriction, but not phenylephrine constriction, in rabbit DA rings. S1QEL did not inhibit mitochondrial metabolism or ETC Complex I activity [69]. Moreover, in human DASMC, S1QEL and rotenone inhibited oxygen-induced increases in cytosolic calcium, a surrogate for DA constriction [69].

In future studies of oxygen sensing the use of these electron leak suppressors should be considered.

Oxygen sensing in the ductus arteriosus and fetal PA

The DA is a vital fetal vessel that shunts placentally oxygenated blood from the PA to the aorta, bypassing the unventilated fetal lung. The simultaneous, opposing, O_2-responses of the fetal PA and DA at birth achieve two goals required for transition from placentally oxygenated fetus to air-breathing neonate: 1) perfusion of newly ventilated lungs and 2) elimination of right to left shunting. This *miracle of birth* occurs flawlessly in term infants but often fails in prematurity, promoting congenital heart diseases, such as patent ductus arteriosus and persistent pulmonary hypertension of the newborn (PPHN). The PA and DA have different embryologic origins, mesodermal lung buds vs left 6th branchial arch, respectively, but we do not fully understand the molecular basis for their opposing O_2-responses. Although functional DA closure is modulated by the endothelium (reinforced by withdrawal of vasodilatory prostaglandin E[70] and increases in the vasoconstrictor, endothelin-1 [71]), human DAs constrict to O_2 in the absence of endothelium [72] and despite blockade of the endothelin pathway [48]. The core of the DA's O_2-sensor mechanism resides in DASMC [48,72].

In unpublished, preliminary data we examined the opposing effects of oxygen on intracellular calcium (a surrogate for vascular tone) in fetal DA vs PA SMC harvested from term rabbits measured using confocal imaging and the calcium indicator, Cal-520AM. These DASMC and PASMC were isolated from the same rabbit kits and grown under identical hypoxic conditions, (PO_2~40 mmHg), to mimic the fetal oxygen condition) (Fig. 3). DASMC respond to normoxia with a rise in $[Ca^{2+}]_i$, which triggers contraction, whilst PASMC have a fall in $[Ca^{2+}]_i$, favouring relaxation (Fig. 3). It is noteworthy, although only a preliminary finding, that elimination of NDUFS2 (achieved using siRNA) inhibits the effects of oxygen in fetal DASMC without altering the effects on fetal PASMC, perhaps indicating different mitochondrial sensors within these two types of SMC (Fig. 3).

This opposing response to oxygen is nicely imaged in a preliminary microCT study of fetal rabbits, which demonstrates that within 15 minutes of birth (and breathing oxygen) the DA is functionally occluded by vasoconstriction while the adjacent pulmonary circulation is vasodilated (Fig. 4). We speculate that this spatial heterogeneity in O_2-responses in adjacent fetal arteries, which is intrinsic to their respective SMCs, reflects mitochondrial heterogeneity. The term *"mitochondrial spatial heterogeneity"* refers to differences in quantity and/or structure of mitochondrial proteins between fetal PA and DA. The fact that ETC inhibitors and redox agents mimic hypoxia and exert opposing effects on both ROS production and vascular tone in the DA and PA, further supports the contention that mitochondrial spatial heterogeneity underlies opposing PA vs DA responses.

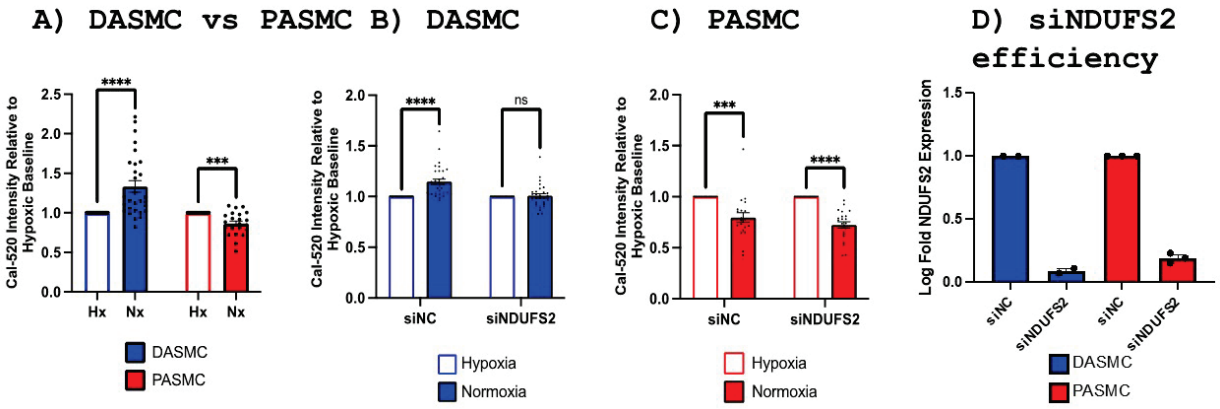

Fig. 3. Oxygen increases $[Ca^{2+}]_i$ DASMC and lowers $[Ca^{2+}]_i$ in PASMC in term, fetal rabbits. **A)** Oxygen increases cytosolic calcium in fetal rabbit DASMC and reduces cytosolic calcium in fetal rabbit PASMC, isolated from the same kit. **B)** Oxygen-induced increases in cytosolic calcium in fetal DASMC are inhibited by small inhibitory RNA (siRNA) targeting NDUFS2. **C)** Oxygen-induced reductions in cytosolic calcium in fetal PASMC are not affected by siNDUFS2. **D)** siNDUFS2 was equally effective in reducing NDUFS2 mRNA expression in both cell types.
**, *** $p<0.01$ and <0.001, respectively. These preliminary data support a role for NDUFS2 as a DA O_2-sensor but also suggests that fetal PASMC may have an alternate sensor for normoxia-induced PA vasodilation. The two blue bars in panel B do not significantly differ from each other statistically; further supporting the interpretation of this preliminary study as showing siNDUFS2 does not alter the oxygen induced fall in PASMC calcium. n=1 DASMC and 1 PASMC cell culture from 1 rabbit kit; data points are individual cells.

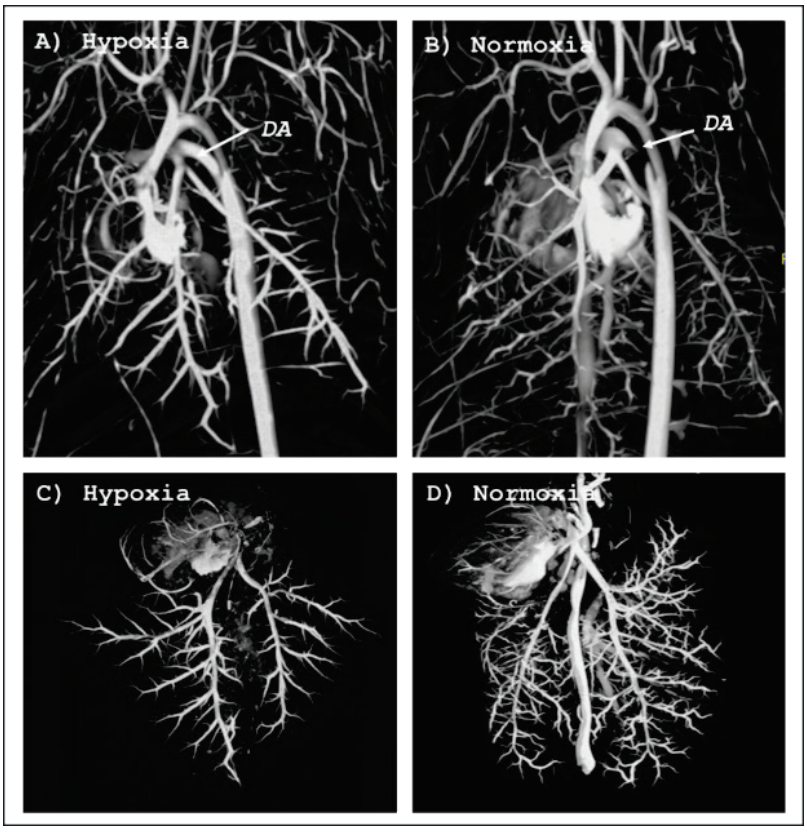

Fig. 4. Contrast-enhanced microcomputed tomography (micro-CT) demonstrates the opposing effects of oxygen on the DA *vs* the pulmonary circulation in newborn, term rabbits. **A-B** *In situ* maximum intensity projection (MIP) micro-CT images taken after perfusion of fetal rabbit kits with an intravascular contrast agent (a mixture of 25 % barium sulfate and 3 % gelatin), via injection of the agent into the beating left ventricle. In panel **A**, acquired during hypoxia, the DA is widely patent (connecting the PA to the aorta). The fetal DA is <0.5 mm in diameter. In panel **B,** acquired within 20 minutes of breathing 100 % O_2, the DA is constricted and functionally closed. **C-D** *Ex vivo* MIP micro-CT images after dissection of heart and lungs from the same (**C**) hypoxic and (**D**) normoxic rabbit kits, indicating the perfusion of the newly ventilated lung vasculature and pulmonary circulation. Note the vasodi-latation of the pulmonary circulation in normoxia. Micro-CT scans were conducted using VECTor^{4}CT (MILabs B.V., Utrecht, Netherlands) preclinical tri-modality ima-ging platform.

In contrast, downstream portions of the O_2-sensing pathways are similar in PA and DA. For example, Kv channel inhibitors, like 4-aminopyridine, cause concordant vasoconstriction in both PA and DA [5,36], whilst Ca_L inhibitors cause concordant vasodilatation [73,74]. Moreover, PA [25] and DA [36] utilize similar ion channels for O_2-responses, including Kv1.5 and Kv2.1.

HPV in lung diseases

Pneumonia, atelectasis HPV optimizes systemic PO_2 in people with atelectasis, pneumonia, COPD, and asthma by optimizing V/Q mismatch and reducing intrapulmonary shunting (Table 1, reproduced with modification from [68]). In these conditions, HPV decreases perfusion of the hypoxic lung segments, shunting blood to better oxygenated lung segments. HPV rapidly reverses upon resolution of pneumonia or once atelectasis resolves (as occurs upon removal of a mucous plug or with lung expansion by incentive spirometry). The intensity of HPV varies between individuals and is reduced in certain diseases. HPV can also be reduced or augmented by medications (Table 1). Calcium channel blockers, like nifedipine or verapamil, reduce HPV [75]. This can result in hypoxemia due to increased intrapulmonary shunting. For example, patients with WHO Group 3 pulmonary hypertension due to COPD, when given nifedipine (20 mg) suffered a fall in arterial PO_2 (52 to 47 mmHg) related to impaired V/Q matching [75]. In contrast, inhaled NO, though a potent pulmonary vasodilator, does not reduce, and may even enhance V/Q matching, because this gas only enters well-ventilated lung segments (where HPV is not active). In contrast, intravenous vasodilators usually decrease V/Q matching by relaxing PAs that perfuse poorly ventilated lung segments [76]. In an *ex vivo* perfused, rabbit lung model, Walmrath *et al.* showed that while intravenous PGI_2 reduced mean PA pressure, it also increased shunt fraction (to ~60 %). In this same study, inhaled NO caused a similar beneficial decrease in mPAP but did not impair V/Q matching (i.e., the shunt fraction remained ~25 %) [76].

Impaired HPV in COVID-19 pneumonia

Our group showed that infection with a murine coronavirus, MHV-1, cause a murine pneumonia which mimics SARS-CoV-2 pneumonia in patients [3]. The suppression of HPV and systemic hypoxemia in this mouse model can be partially reversed by administration of the Ca_L opener, BAYK8644 (Fig. 5). HPV is also suppressed in patients with COVID-19 pneumonia [77].

Table 1. The role of HPV in respiratory diseases

Disease	Role of HPV	Treatment	References
High altitude pulmonary edema (HAPE)	Exaggerated HPV; over perfusion of non-constricted pulmonary vasculature	The calcium channel blocker nifedipine reduces the incidence of HAPE from 63 to 10 %	[78,79,92,93]
Asthma	HPV maintains V/Q balance during acute bronchoconstriction	Intravenous isoprenaline decreases mPAP in an isolated perfused rat lung model of asthma (by 3-5 mmHg)	[92,94-96]
Chronic obstructive pulmonary disease (COPD)	HPV is reduced, however the residual HPV response improves V/Q balance and systemic oxygenation	In COPD patients Almitrine (100mg/day), a drug that enhances HPV, increases PaO_2 from 52 to 59 mmHg. In COPD patient with pulmonary hypertension and respiratory failure prostacyclin IV is a nonselective vasodilator that causes mild hypoxemia.	[92,97-99] [100]
Pneumonia	Diversion of blood flow away from the diseased lobe(s) of lung optimizes systemic PO_2	Pneumonia impairs HPV in experimental models. In COPD patients with hypoxic exacerbations, intravenous prostaglandins increase systemic hypoxemia, consistent with the adverse effects of suppressing HPV	[92,97,100-102]
Acute Lung Injury (ALI)/Acute Respiratory Distress Syndrome (ARDS)	HPV is reduced in ALI however the residual HPV improves V/Q balance and reduces shunting	In an ovine model of ARDS inhibition of inducible NOS restores HPV, increases PaO_2/FiO_2, and decreases shunt fraction	[92,97,103,104]
COVID-19 pneumonia	HPV is suppressed in an MHV-1 murine model of COVID-19 pneumonia HPV is also suppressed in patients with COVID-19 pneumonia [77]	BAYK8644 (an opener of Ca_L channels) enhances HPV	[3]

Modified and reproduced from [68]

We showed that the SARS-CoV-2 virus (and its proteins) directly attack the mitochondria in human and rodent PASMC, leading to impaired oxygen sensing and reduced HPV [3]. Indeed, this effect is seen with other coronaviruses too, including HCoV-OC43 and MHV-1. SARS-CoV-2 infections downregulate expression of ETC Complex I and ATP synthase genes and upregulate apoptosis-inducing genes. In additional to the adverse transcriptional effects of coronaviruses on mitochondria we demonstrated direct protein-protein interactions between viral proteins and key components of the mitochondrial ETC that are relevant to oxygen sensing. For example, in normal human PASMC, lentiviral transduction with SARS-CoV-2's M or Nsp9 proteins inhibits HPV [3]. This is SARS-CoV-2 mitochondriopathy is directly relevant to HPV in vivo. For example, in a murine model of COVID-19 pneumonia, created by infection of mice with the murine coronavirus MHV-1, HPV is suppressed and a ventilation-perfusion mismatch lowers systemic oxygenation, much as occurs in patients with COVID-19 pneumonia [77]. BAY K8644, a calcium channel agonist, increased HPV and improved systemic oxygenation in this murine COVID pneumonia model. This SARS-CoV-2 mitochondriopathy also causes mitochondrial apoptosis in airway epithelial cells, contributing to diffuse alveolar damage in COVID-19 pneumonia (illustrated in Fig. 6) [3].

Excessive HPV in High Altitude Pulmonary Edema (HAPE)

While HPV is largely beneficial, excessive and spatially heterogenous HPV can be harmful, as is the case

in HAPE [78,79] (Table 1). Rapid ascent to high altitude without acclimation triggers a non-cardiogenic pulmonary edema that manifests as cough, dyspnea, and reduced exercise performance, within 2-5 days of ascent to altitudes exceeding 2500 m [80]. In people with HAPE, excessive HPV stresses and distends the more proximal arterial walls ultimately rupturing the basement membrane and disrupting the alveolar-capillary barrier [81]. Hypoxia also inhibits alveolar fluid clearance within the lung by inhibiting sodium exchangers, thereby decreasing sodium transport and reducing the lung's ability to reabsorb fluid [81]. Evidence that excessive HPV promotes HAPE comes from a study of simulated rapid ascent to altitude. Dehnert et al found that individuals with the strongest HPV response are most at risk of HAPE. They exposed 421 healthy Caucasians to hypoxia (simulating their elevation to 4500 m over 24 hours). 13 % of subjects with exaggerated HPV (systolic PA pressure in hypoxia 51±6 mmHg on echo Doppler, n=4/39) developed HAPE within 48 hours. Subjects with milder HPV (systolic PA pressure at altitude of 33±5 mmHg) did not develop HAPE [82]. Likewise, in a rabbit model of ascent to altitude, suppression of HPV (using acetazolamide) reduced HAPE [83]. HAPE is also reversible with supplemental oxygen and rapid descent from altitude [84] and in the short term can be treated or prevented by inhibitors of HPV, such as nifedipine or sildenafil, while descent is in progress [85].

Pulmonary hypertension

There is an intersection between pulmonary arterial hypertension (WHO Group 1 PH) and HPV. In some regards, the pulmonary circulation in people or animals with Group 1 PH behaves as if there were a failure of oxygen sensing, meaning that despite normal FiO_2 pathways are activated as if the lung were hypoxic. We refer to this normoxic elevation of pulmonary vascular tone, with downregulation of oxygen-sensitive Kv channels and normoxic activation of HIF-1α, as a state of *pseudohypoxia*, reviewed in [86]. A human condition exemplifying the role of dysregulated oxygen sensing in pulmonary hypertension was brought to our attention by our late colleague, Dr John Newman, a pioneering expert in high altitude pulmonary

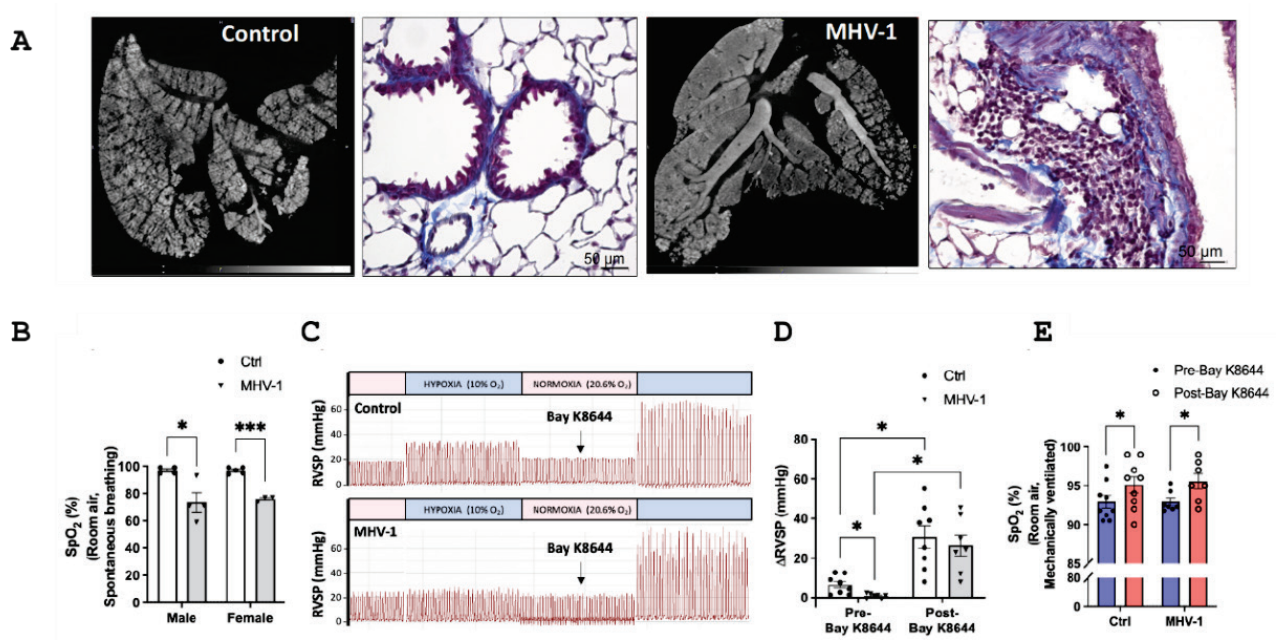

Fig. 5. Suppression of hypoxic pulmonary vasoconstriction in a murine coronavirus model of COVID-19 pneumonia. Loss of HPV may contribute to hypoxemia in COVID-19 pneumonia. Here the effects of coronavirus infection on HPV are studied in a murine model created by MHV-1 infection at days 4–6. (n=4 for male Ctrl, n=4 for male MHV-1, n=5 for female Ctrl, n=3 for female MHV-1). Micro-CT images acquired using a VECTor^{4}CT scanner. The CT scans show lung consolidation in MHV-1 infected mice. The histology insets (on right) show inflammatory infiltrates in the MHV-1 infected lungs. Reduction in oxygen saturation occurs in both male and female mice with MHV-1 pneumonia. **C)** Representative RV pressure traces and mean data show that HPV is suppressed in MHV-1 mice. There was no significant increase in RV systolic pressure with hypoxia in MHV-1 infected mice, in contrast to the robust rise in RVSP (HPV) in uninfected mice. **D)** Intraperitoneal treatment with the CaL agonist BAYK8644 significantly augmented HPV (defined as the increase in RVSP in response to hypoxia) in both control and infected mice. (n=8 for Ctrl, n=7 for MHV-1). **E)** Bay K8644 (1mg/kg IP) increases O_2 saturation. (n=9 for Ctrl, n=7 for MHV-1). The higher O_2 saturations in this panel (vs panel **B**) reflect the fact these mice were being mechanically ventilated with room air (versus spontaneous breathing of room air in panel **A**). **P*<0.05, ***P*<0.01, ****P*<0.001. Reproduced with permission from [3].

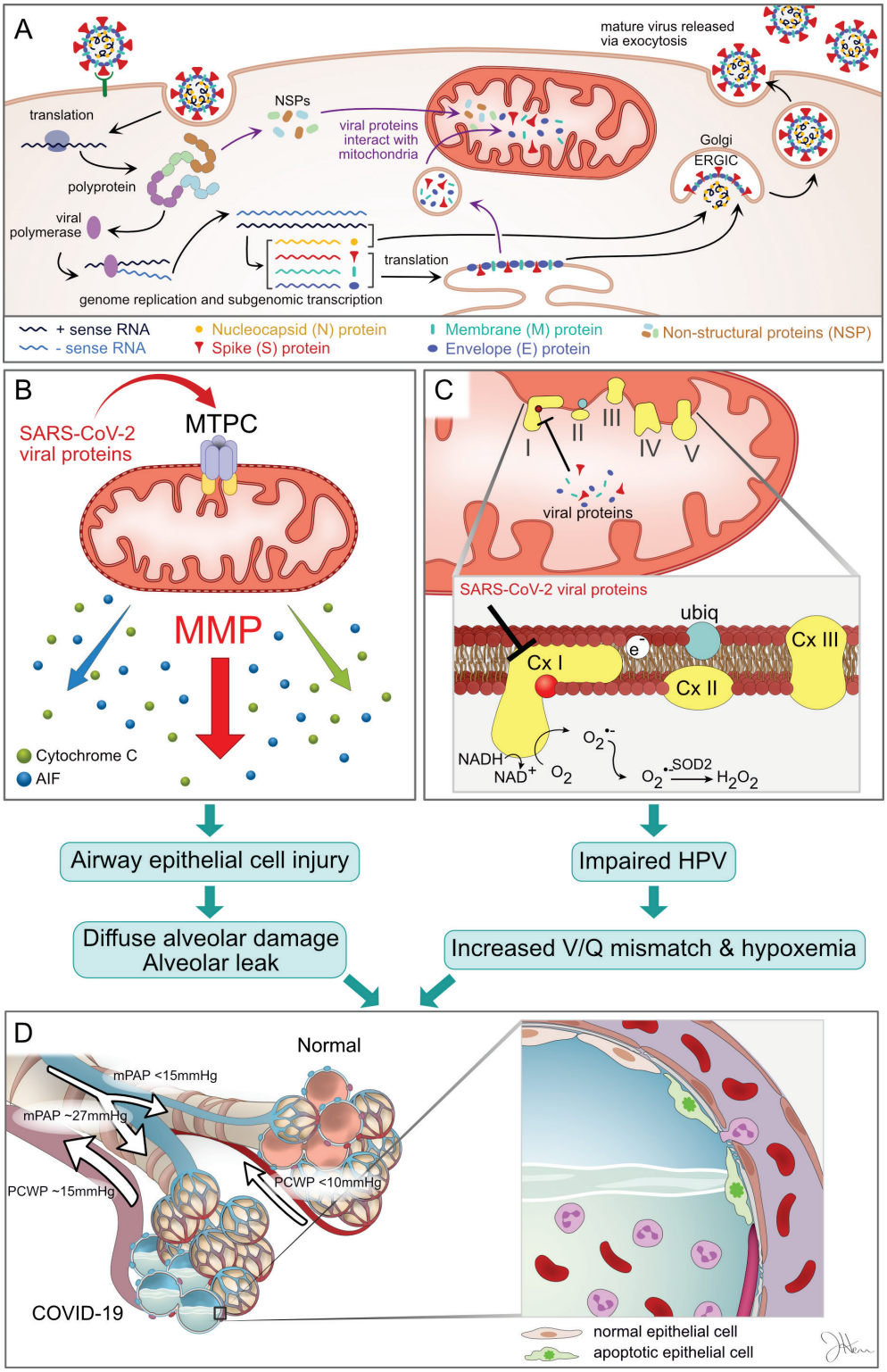

Fig. 6. Proposed mechanism by which SARS-CoV-2 mitochondriopathy contributes to hypoxemia in COVID-19 pneumonia. SARS-CoV-2 mitochondriopathy causes diffuse alveolar damage (DAD) and hypoxemia in part by inducing apoptosis (left) in airway epithelial cells and in part by suppressing HPV in PASMC (right)**. A)** Viral replication floods the cell with viral proteins. **B)** In airway epithelial cells some viral proteins target the mitochondria transition pore complex (MTPC) proteins which permeabilizes the membrane (MMP) and leads to leak of apoptosis mediators, like AIF and cytochrome *c*, resulting in apoptosis. **C)** In PASMC viral proteins interact with components of ETC Complex I, the site of O_2-sensing, and impair HPV. **D)** The consequences of this mitochondriopathy include impaired HPV, which inappropriately floods capillaries in infected segments with blood, exacerbating capillary leak by promoting V/Q mismatch. Excessive apoptosis damages the alveoli and contributes to DAD. The loss of HPV combined with AEC apoptosis exacerbate systemic hypoxemia in coronavirus pneumonia syndromes, including COVID-19 pneumonia. In addition, there is profound mitochondrial fission and bioenergetic impairment which contribute to the lung injury (not shown in this figure). Reproduced with permission from [3].

hypertension and respirologist who worked at Vanderbilt University [87]. After hearing of the impaired oxygen sensing and spontaneous pulmonary hypertension in fawn-hooded rats (FHR), which relates to their normoxic activation of HIF-1α [88], Dr. Newman acquainted our group with Chuvash disease. People with Chuvash disease, named for a mid-Volga River region in Russia, have enhanced HPV, polycythemia and pulmonary hypertension, despite normal inspired oxygen levels [89]. Chuvash disease results from a missense mutation within the von Hippel-Lindau (VHL) gene which impairs VHL's ability to interact with the α-subunits of hypoxia inducible factors (HIF-1α and HIF-2α). Normally, HIF is hydroxylated by prolyl hydroxylases, marking these proteins for ubiquit-ination by VHL, which ultimately leads to their proteasomal degradation. The Chuvash VHL mutation impairs this degradation pathway resulting in normoxic HIF stabilization and paradoxical normoxic transcription of HIF-regulated genes during normoxia, including erythropoietin [89-91]. Thus, patients with Chuvash PH are afflicted by a failure of oxygen sensing and aspects of this are seen in in patinets with WHO Group 1 PH.

FHR, like Chuvash patients, develop mild polycythemia with age and manifest normoxic activation of HIF-1α. However, in FHR the syndrome does not relate to a VHL mutation; rather the state of pseudohypoxia reflects epigenetic silencing of mitochondrial SOD2 by DNA methyltransferase [10]. Interestingly in FHR, as in residents of high altitude, the signal transduction of acute HPV is downregulated, meaning that despite adverse pulmonary vascular remodeling and pulmonary hypertension the acute response to hypoxia (the magnitude of HPV) is depressed in FHR, compared to control rats.

It is a pleasure to contribute this review of the mechanisms of oxygen sensing to an issue of the journal published in honor of Dr. Jan Herget. Jan had an intense interest in the effects of hypoxia on the pulmonary vasculature, both acute and chronic. Over 30 years, between 1985 and 2015, he was an author on 21 papers dealing with the pathophysiologic mechanisms involved, in both perinatal and adult animals. In most he was the first or senior author. The mechanisms he studied involved the study of potassium channels, reactive oxygen species and nitric oxide. In addition to his research, Jan organized a series of international pulmonary hypertension meetings in Prague. These took place both before and after the fall of the Berlin wall and were the premiere venue in which to present and discuss pulmonary hypertension research. He was always amazingly hospitable to clinicians and scientists from all over the world.

Conclusion

The homeostatic oxygen sensing system plays a vital role in adapting humans, and most mammalian species, to reduced oxygen availability (hypoxia) which they experience in utero, at altitude and during disease. The oxygen sensing mechanisms found in PASMC, DASMC and type 1 cells of the carotid body are largely based in the mitochondrial electron transport chain and are mediated by ETC subunits, including NDUFS2. Changes in PO_2 rapidly alter production of mitochondrial reactive oxygen species, including hydrogen peroxide, which in turn regulate the opening of redox-sensitive potassium channels. The K+ channels regulate membrane potential and thereby control calcium influx through voltage-gated calcium channels. In PASMC hypoxia induces a fall in ROS production which inhibits Kv1.5 and other Kv channels, thereby activating Ca_L channels and initiating vasoconstriction. There is a diversity in the types of mitochondria found in the PASMC versus systemic arterial SMC such that PASMC respond uniquely to hypoxia. Likewise, there is regional heterogeneity in the expression and activity of oxygen-sensitive K+ channels along the length of the pulmonary circulation, such that they are enriched in small resistance PAs, the site of HPV. HPV remains as fascinating a subject of study in 2024 as it was in 1894.

Conflict of Interest

There is no conflict of interest.

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