Published in final edited form as:
Curr Opin Pharmacol. 2009 June ; 9(3): 287–296. doi:10.1016/j.coph.2009.02.006.

Mechanisms of hypoxic pulmonary vasoconstriction and their roles in pulmonary hypertension: new findings for an old problem

Jeremy P.T. Ward[1] and **Ivan F. McMurtry**[2]

1 *King's College London, Division of Asthma, Allergy and Lung Biology, London SE1 9RT, UK*

2 *University of South Alabama College of Medicine, Department of Pharmacology and Centre for Lung Biology, Mobile, AL 36688-0002, USA*

Abstract

Hypoxic pulmonary vasoconstriction (HPV) normally optimises ventilation-perfusion matching in the lung, but leads to pulmonary hypertension under conditions of global hypoxia. The past few years have provided some major advances in our understanding of this complex phenomenon, but significant controversy remains concerning many of the key underlying mechanisms. On balance, recent evidence is most consistent with an elevation in mitochondria-derived reactive oxygen species as a key event for initiation of HPV, with consequent Ca^{2+} release from intracellular ryanodine-sensitive stores, although the activation pathways and molecular identity of the associated Ca^{2+} entry pathways remain unclear. Recent studies have also raised our perception of the critical role played by Rho kinase in both sustained HPV and the development of pulmonary hypertension, further promoting Rho kinase and the pathways regulating its activity and expression as important therapeutic targets.

Introduction

Hypoxic pulmonary vasoconstriction (HPV) is an important physiological mechanism that optimises ventilation-perfusion matching and pulmonary gas exchange by diverting blood flow from poorly ventilated areas of the lung. However, in conditions associated with global hypoxia, such as respiratory disease or altitude, HPV leads to detrimental increases in pulmonary artery (PA) pressure. In chronic hypoxic lung disease dysfunction of mechanisms regulating vascular tone and remodelling of the pulmonary vasculature contribute to the development of sustained pulmonary hypertension (PH). The mechanisms underlying HPV and their role in the development of chronic PH remain incompletely resolved. This review focuses primarily on literature from the past 2 years, that exemplify new insights and some of the current controversies surrounding this complex phenomenon.

Correspondence: Professor Jeremy PT Ward, Ph.D., King's College London, Academic Centre, Henriette Raphael House, Guy's Campus, London SE1 1UL, UK, Tel/fax: +44 020 7848 6695, E-mail: jeremy.ward@kcl.ac.uk, Professor Ivan F. McMurtry, Ph.D., Department of Pharmacology, MSB 3316, 307 N. University Blvd, University of South Alabama, Mobile, AL 36688-0002, USA, e-mail: E-mail: ifmcmurtry@usouthal.edu.

Characteristics of HPV

The primary locus of HPV is the PA smooth muscle cell (PASMC), although the endothelium plays an important facilitatory or permissive role for sustained HPV. Whilst acute hypoxia often causes a monophasic increase in PA pressure *in vivo*, the response in isolated PA is biphasic (also observed in some perfused lung and *in vivo* studies). This consists of a rapid, transient vasoconstriction lasting about 10–15 min (phase 1), and a slowly developing vasoconstriction that is sustained as long as hypoxia is present (phase 2) (e.g. [1,2, reviewed in 3]; Figure 1). Differing mechanisms underlie these components. Whilst both are associated with an elevation of PASMC intracellular [Ca^{2+}] ([Ca^{2+}]$_i$), variously attributed to voltage-dependent and -independent Ca^{2+} entry, Ca^{2+} release from ryanodine-sensitive intracellular stores and/or capacitative or store operated Ca^{2+} entry (SOCE), the sustained phase is also highly dependent on RhoA/Rho kinase (ROCK) mediated Ca^{2+} sensitisation (reviewed in [3]).

Initiation of HPV

PASMC mitochondria act as the O_2 sensor and initiate HPV, but the downstream signalling pathways remain controversial [4,5]. Whereas the Redox hypothesis proposes that decreased generation of mitochondrial reactive oxygen species (ROS) and a more reduced cytosolic redox state initiates HPV [6], the ROS hypothesis proposes the response is due to increased generation of ROS [7]. Alternatively, it has been suggested that hypoxia elevates the cytosolic AMP/ATP ratio which activates AMP-activated kinase (AMPK) and stimulates production of cyclic ADP ribose (cADPR), an endogenous activator of ryanodine receptors [8] (See Figure 1).

Recently, Mehta *et al*. [9] reported that hypoxia (≥1hr) decreased ROS generation in human cultured PASMC, as estimated with 3 different probes. This study is interesting in that the hypoxic challenge was longer than most, and coronary artery smooth muscle cells responded in a qualitatively similar fashion to PASMC, implying that differences in the response to hypoxia between PA and systemic arteries relate to downstream effectors rather than O_2 sensing pathways. Similar results are reported by Wu *et al.* [10] in the same tissues, though they also showed that ROS were increased by endothelin-1 and after 48 hr of hypoxia. Indirect support for the Redox hypothesis also comes from a study showing that the thiol oxidant diamide causes PA vasodilatation via activation of K^+ channels and inhibition of SOCE [11]. Whereas oxidising agents have previously been shown to activate K^+ channels, this is the first report to show inhibition of SOCE, a key component of HPV.

Although the above are consistent with several previous studies (reviewed in [5,6]), they differ from numerous other recent reports. Hypoxia has been reported to increase mitochondrial ROS production in both freshly isolated and cultured rat PASMCs, albeit over shorter durations of hypoxia [12,13], and the same result was obtained using a novel FRET based redox probe [5, 14], which does not have the serious limitations of commonly-used probes (see [15]). Indirect evidence for the ROS hypothesis is also provided by recent observations that low concentrations of peroxide induce sustained constriction of PA [16] and elevation of PASMC [Ca^{2+}]$_i$ via release from ryanodine sensitive stores [12,13,16,17]. Moreover, antioxidants or overexpression of antioxidant mechanisms suppress HPV in perfused lungs [18] and hypoxia-induced elevation of [Ca^{2+}]$_i$ in PASMC [12]. ROS have also been implicated as mediators of ROCK activation in chronic hypoxic PH [19]. Intriguingly, Rathore *et al*. have added another level of complexity by showing in PASMC that the hypoxia-induced elevation in mitochondrial ROS generation activates NADPH oxidase via PKCε, consequently enhancing the levels of ROS production and [Ca^{2+}]$_i$ [13], though it has been noted that this mechanism of ROS-induced ROS production apparently only applies to the initial transient phase of HPV [20] (Figure 1).

Whereas on balance recent reports strongly favour the ROS hypothesis, Robertson *et al.* [21] have provided additional evidence for the AMPK hypothesis, by showing that compound C, an inhibitor of AMPK, suppresses both hypoxia-induced phosphorylation of a classical AMPK substrate and HPV in PA. However, this highly suggestive report is inconclusive due to the recently reported non-selectivity of Compound C [22].

K_V channels and HPV

It has been proposed that inhibition of voltage-gated K^+ channels (K_V) channels, particularly $K_V1.5$, plays a central role in HPV (see [6], Figure 2), although this has been questioned (see [3]). Whilst the Redox hypothesis proposes that this inhibition is caused by reduction of K_V channel residues (see above), an intriguing study by Cogolludo *et al.* [23] has implicated a novel pathway involving increased production of ceramide by neutral sphingomyelinase and subsequent activation of PKCξ, which inhibits K_V channels.

Platoshyn *et al*. [24] have demonstrated that K_V expression in PASMC is heterogeneous, and that hypoxia only inhibits IK_V in cells expressing high levels of $K_v1.5$ (46% of cells), and elicits Ca^{2+} release from cyclopiazonic acid (CPA)-sensitive stores in ~34% of PASMC (see below). The authors speculate that in HPV this sub-set of hypoxia-sensitive cells signal to the remainder via gap junctions. Notably, a theoretical model has suggested that hypoxic inhibition of K^+ currents is insufficient to cause contraction, unless combined with activation of non-selective cation channels (NSCC) [25]. This is potentially a key finding, as NSCC, possibly associated with SOCE (see below), have been strongly implicated in HPV (reviewed in [3], Figure 2). Interestingly, Firth *et al*. [26] have described a novel Mg^{2+} and ATP-mediated mechanism by which mitochondria may directly influence K_V activity, in addition to any effects of ROS or redox state, such that inhibition of oxidative phosphorylation increases IK_V at negative potentials, but decreases IK_V at positive potentials. Both studies predict that inhibition of IK_V by hypoxia may only occur following partial depolarisation, as has been observed in several previous studies (reviewed in [4]). Importantly, in a subsequent study Firth *et al*. [27] show that this mechanism is facilitated by a very close approximation of mitochondria with the PASMC membrane, but that in systemic artery smooth muscle this close approximation is absent. This discovery of a difference in the spatial distribution of mitochondria has potentially important ramifications for all types of mitochondrial signalling in PASMC.

Ca^{2+} entry and release pathways

Ca^{2+} release from ryanodine sensitive stores is generally regarded as obligatory for HPV (reviewed in [3], Figure 2), Li *et al*. [28] have now demonstrated that homo- and heterozygous gene deletion of RyR1 in mice blocks the hypoxia-induced elevation of $[Ca^{2+}]_i$ in PASMC and HPV in PA. Intriguingly, depolarisation-induced PA contraction and elevation of $[Ca^{2+}]_i$ in both Ca^{2+} free and containing media were also substantially suppressed in RyR1+/− mice [28], implying an unexpected link between depolarisation and ryanodine-sensitive stores. Mouse resistance PA are reported to have about equal expression of RyR1, 2 and 3, compared to conduit PA and mesenteric arteries which have low expression of RyR3 [29]. Interestingly, Kinnear *et al.* [30] have reported spatial heterogeneity of RyR within rat PASMC, with RyR3 predominant in the perinuclear region, and provide intriguing evidence that clustering of lysosomes and RyR3-rich SR in this region provides a trigger zone for NAADP (nicotinic acid adenine dinucleotide phosphate)-induced Ca^{2+} signalling and generation of Ca^{2+} waves. This and the spatial distribution of RyR subtypes could have important ramifications for Ca^{2+} signalling in HPV.

Depletion of Ca^{2+} stores activates SOCE, which is strongly implicated in HPV (reviewed in [3]). However, ryanodine differentially suppresses phase 2 of HPV [31], whilst the SERCA

(sarco-endoplasmic reticulum Ca^{2+} ATPase) inhibitor CPA only inhibits phase 1, and the pharmacological profile of voltage-independent Ca^{2+} entry differs between phases [32]. Notably, CPA does not affect caffeine-induced Ca^{2+} release in canine freshly isolated PASMC, but abolishes that induced by agonists, implying that IP_3- and ryanodine-sensitive stores are functionally separate [33]. Ng *et al.* [34] have also found in the same preparation that whereas SOCE elicited by store depletion with CPA and agonists requires functional IP_3 receptors, hypoxia-induced SOCE does not, and concluded that mechanisms in addition to store depletion are involved in the latter [34]. These and other data suggest that more than one SOCE pathway exists (e.g. [35]), possibly linked to different stores.

However, Ng *et al*. [33] have reported that culture of PASMC reorganises IP_3- and ryanodine-sensitive stores into a single pool, and that both pharmacological depletion of this store and hypoxia activate not only SOCE and voltage-gated Ca^{2+} entry, but also Ca^{2+} entry via reverse operation of the Na^+-Ca^{2+} exchanger (NCX), which was not observed in fresh cells [33]. This pooling of CPA- and ryanodine-sensitive stores presumably reflects the finding of Platoshyn *et al*. [24] that CPA abolishes hypoxia-elicited Ca^{2+} release in cultured PASMC (see above). Another study also excludes a role for reverse operation of NCX in HPV of isolated PA [36], suggesting that NCX may be upregulated during dedifferentiation and proliferation of PASMC [33], as recently observed in PASMC from patients with idiopathic pulmonary arterial hypertension (IPAH) [37]. Under these conditions Na^+ influx via NSCC associated with SOCE (see below) could be responsible for activating reverse mode NCX [33] Figure 2). An important message is that culture alters Ca^{2+} handling in PASMC, making this model of limited use for the study of hypoxia-induced mobilisation of Ca^{2+} in HPV.

Until recently, the molecular correlates of SOCE were generally believed to be NSCC formed of TRPC proteins, but this is being reconsidered following the discovery that the Ca^{2+} release activated channel (CRAC) is due to interaction between the endoplasmic reticulum Ca^{2+} sensor STIM (stromal-interacting molecule) and Orai channel proteins [38]. However, CRAC is highly Ca^{2+} selective, whereas store depletion- and hypoxia-induced SOCE has been associated with NSCC in PASMC (see [34,39]). Recent reports suggest that STIM1 may interact with most TRPC proteins to determine their function as store-operated channels [40]. Interestingly, Lu *et al*. [41] have shown that the amplitude of both store depletion- and hypoxia-induced SOCE correlates with expression of STIM1, TRPC1, TRPC4 and TRPC6, all being much greater in PASMC from distal PA which constrict most strongly to hypoxia. Notably, IPAH has been associated with enhanced SOCE and expression of TRPC3 and TRPC6 [42].

Direct evidence for a role for TRPC6 in HPV has been provided by Weissmann *et al.* [2], who showed that phase 1 of HPV and the associated elevation of PASMC $[Ca^{2+}]_i$ were ablated in TRPC6−/− mice, and that regional hypoventilation caused severe hypoxaemia in these animals. The latter also demonstrates that phase 1 of HPV is important for acute optimisation of regional gas exchange. However, neither phase 2 of HPV nor chronic hypoxia-induced PH were affected, suggesting that TRPC6 plays little role in SOCE activated by depletion of ryanodine sensitive stores (see above). Notably, hypoxia increased diacylglycerol, an activator of TRPC6, in PASMC, which could be due to ROS-mediated activation of PLC [2] (Figure 2). Moreover, Keseru *et al*. [43] report that hypoxia causes translocation of TRPC6 to the membrane, and provide evidence that this is mediated by cytochrome P450-derived epoxyeicosatrienoic acids (EETs).

Ca^{2+} sensitisation and Rho kinase

ROCK-mediated Ca^{2+} sensitisation of PA contraction is of critical importance for sustained HPV (reviewed in [3]), and contributes to increased pulmonary vascular resistance (PVR) in chronic hypoxic PH [44–46] (Figure 3). However, Wang *et al.* [47] add a twist to the story, at

least in normotensive PA, by reporting that inhibition of ROCK or MLCK also suppresses the hypoxia-induced elevation in $[Ca^{2+}]_i$, possibly via disruption of cytoskeletal-dependent spatial interrelationships among the signalling components. Whether these are promoted by activation of ROCK or sustained by basal ROCK activity (which is significant in PA [48,49]) remains to be determined. The mechanism by which hypoxia activates ROCK has until recently remained obscure. Knock *et al*. have now shown in rat distal PA that Src-family kinases are involved in the activation of ROCK by both agonists [48] and hypoxia [1], and indeed also by superoxide (but not peroxide) [50], consistent with the ROS hypothesis of HPV (Figure 3). Notably, basal activity of ROCK is independent of Src [1,48]. There is evidence that stimulation of ET_A and $5\text{-}HT_{1B/1D}$ receptors by endogenous ET-1 and 5-HT is involved in sustained activation of ROCK in chronically hypoxic hypertensive rat PA [51], and this might also be mediated by increased production of ROS [19].

Factors other than ROCK can attenuate myosin phosphatase activity and thereby augment myosin phosphorylation. Telokin is a myosin-binding protein homologous with the C-terminal domain of MLCK, and when phosphorylated promotes myosin phosphatase activity [52] (Figure 3). An intriguing study by Madden *et al*. [53] suggests that in normoxia levels of phosphorylated telokin are greater in feline distal PA compared to arteries that relax to hypoxia, but that in hypoxia telokin is dephosphorylated, thus facilitating myosin phosphorylation and contraction. The importance of this phenomenon is unknown, though it has implications for the mechanism of action of cGMP-raising agents such as sildenafil, as telokin is a substrate for protein kinase G-mediated phosphorylation [52].

Although acute inhibition of ROCK reverses the sustained increase in PVR in chronic hypoxic models of PH [44,45], there is debate concerning the role of vasoconstriction in severe occlusive PH, where the increase in PVR has been attributed primarily to vascular remodelling. Oka *et al*. [54] utilised a rat model of severe occlusive PH, where chronic hypoxia is coupled with blockade of VEGF receptors, to address this question. The model exhibited occlusive pulmonary vascular lesions, and sustained vasoconstriction that was reversed by acute inhibition of ROCK [54]. Consistent with this, chronic hypoxia induces ROS and ROCK-dependent, Ca^{2+} signal-independent myogenic tone in rat distal PA [55]. In contrast to the apparent Ca^{2+} signal-independent constriction of the adult rat hypertensive PA, Hirenallur-S *et al*. [56] observed increased voltage-gated L-type Ca^{2+} channel-dependent vasoconstriction and upregulation of the pore-forming α_{1C}-subunit in distal PA of chronically hypoxic piglets. ROCK-mediated vasoconstriction also plays a role in the PH of fawn-hooded rats [57]. However, the activation of ROCK in this model might not be due to ROS, since Bonnet *et al*. [58] have reported that ROS levels are diminished in the hypertensive pulmonary arteries of fawn-hooded rats.

The role of ROCK in PH-associated vascular remodelling remains to be deciphered [see [46]). Whereas Mair *et al*. [59] report that ROCK potentiates $5\text{-}HT_{1B}$ receptor stimulated pulmonary fibroblast proliferation and that hypoxic PH exaggerated by overexpression of the human serotonin transporter (SERT) in mice is associated with elevated expression of ROCK and ROCK-dependent vascular remodelling, Desbuards *et al*. [60] have observed in rats that discontinuation of dexfenfluramine treatment augments the severity of subsequent hypoxia-induced PH, and that this is associated with increased activation of RhoA and expression of SERT and ROCK, but no apparent worsening of the PA muscularization. A recent report suggests that upregulation of RhoA and ROCK expression in the remodelled resistance PA of explanted IPAH lungs is associated with activation of a non-canonical WNT-pathway, the planar cell polarity pathway [61].

Conclusions

The studies reviewed here exemplify the heterogeneity of the field, and reaffirm HPV as a complex phenomenon involving a multiplicity of interacting mechanisms. Thus it is often difficult to distinguish key mechanisms from those that are permissive or facilitatory. Moreover, it is now even more apparent that the immediate and sustained components of HPV are different, and that mechanisms specifically involved in the latter play a key role in the development of PH. Nevertheless, the past 2 years have provided some important advances. Whilst irrefutable proof is still lacking, the weight of evidence for an elevation of ROS as the trigger of HPV is now very strong. The key role of ryanodine-sensitive Ca^{2+} stores in HPV is now also firmly established, though further work is required to determine the nature, regulation and molecular correlates of SOCE, particularly the putative interaction between STIM/Orai and TRPC proteins. An increasingly important area concerns ROCK, which has now been identified as central to both sustained HPV and the development of PH (Figure 4). This further affirms ROCK and the pathways leading to its activation and expression as prime therapeutic targets.

Acknowledgments

Work in the authors' laboratories was supported by: the Wellcome Trust (078075) and British Heart Foundation (PG21995) [JPTW]; the National Heart, Lung, and Blood Institute Grant HL-15985 and University of South Alabama [IFMc]. We thank PI Aaronson for commenting on the manuscript.

Abbreviations

5-HT
: serotonin, 5-hydroxytryptamine

AMPK
: AMP activated kinase

cADPR
: cyclic ADP ribose

CaM
: Ca^{2+}-calmodulin

CRAC
: Ca^{2+} release activated channel

DAG
: diacylglycerol

ET-1
: endothelin-1

HPV
: hypoxic pulmonary vasoconstriction

IPAH
: idiopathic pulmonary arterial hypertension

K_V
: voltage gated K^+ channels

L-type
: voltage-gated Ca^{2+} channels

MP
: myosin phosphatase

NAADP
: nicotinic acid adenine dinucleotide phosphate

NCX
: Na^+-Ca^{2+} exchanger

NSCC
: non-selective cation channels

PASMC
: pulmonary artery smooth muscle cell

$PGF_{2\alpha}$
: prostaglandin $F_{2\alpha}$

ROCK
: Rho kinase

ROS
: reactive oxygen species

RyR
: ryanodine receptors

SERCA
: sarco-endoplasmic reticulum Ca^{2+} ATPase

SERT
: serotonin transporter

SFK
: src family kinases

SO
: superoxide

SOC
: store operated channels

SOCE
: store operated Ca^{2+} entry

SR
: sarcoplasmic reticulum

STIM
: stromal-interacting molecule

TRP
: transient receptor potential protein

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:
• of special interest; •• of outstanding interest

1•. Knock GA, Snetkov VA, Shaifta Y, Drndarski S, Ward JP, Aaronson PI. Role of src-family kinases in hypoxic vasoconstriction of rat pulmonary artery. Cardiovasc Res 2008;80:453–462. [PubMed: 18682436]This paper presents the first evidence that src family kinases (SFK) are involved in HPV. HPV in rat intrapulmonary arteries was associated with phosphorylation of src, MYPT-1 and MLC_{20} all of which were inhibited by SFK inhibitors. RNAi knockdown of src also suppressed hypoxia-induced translocation of ROCK. The authors conclude that SFK are key regulators of ROCK in HPV.

2••. Weissmann N, Dietrich A, Fuchs B, Kalwa H, Ay M, Dumitrascu R, Olschewski A, Storch U, Mederos YSM, Ghofrani HA, Schermuly RT, et al. Classical transient receptor potential channel 6 (TRPC6) is essential for hypoxic pulmonary vasoconstriction and alveolar gas exchange. Proc Natl Acad Sci U S A 2006;103:19093–19098. [PubMed: 17142322]This report demonstrates that TRPC6 is of critical importance for the phase 1 component of HPV in perfused lungs and the associated elevation of $[Ca^{2+}]_i$ in PASMC derived from TRPC−/− mice. Notably, neither sustained HPV nor development of chronic hypoxia-induced PH were altered in TRPC−/− mice. The authors suggest that HPV involves stimulation of Ca^{2+} entry via TRPC6-containing channels via an hypoxia-induced elevation of diacylglycerol, possibly via activation of PLC by ROS.

3. Aaronson PI, Robertson TP, Knock GA, Becker S, Lewis TH, Snetkov V, Ward JP. Hypoxic pulmonary vasoconstriction: mechanisms and controversies. J Physiol 2006;570(Pt 1):53–58. [PubMed: 16254010]

4. Ward JP, Snetkov VA, Aaronson PI. Calcium, mitochondria and oxygen sensing in the pulmonary circulation. Cell Calcium 2004;36(3–4):209–220. [PubMed: 15261477]

5. Waypa GB, Schumacker PT. Oxygen sensing in hypoxic pulmonary vasoconstriction: using new tools to answer an age-old question. Exp Physiol 2008;93(1):133–138. [PubMed: 17993507]

6. Moudgil R, Michelakis ED, Archer SL. Hypoxic pulmonary vasoconstriction. J Appl Physiol 2005;98 (1):390–403. [PubMed: 15591309]

7. Waypa GB, Schumacker PT. Hypoxic pulmonary vasoconstriction: redox events in oxygen sensing. J Appl Physiol 2005;98(1):404–414. [PubMed: 15591310]

8. Evans AM. AMP-activated protein kinase underpins hypoxic pulmonary vasoconstriction and carotid body excitation by hypoxia in mammals. Exp Physiol 2006;91(5):821–827. [PubMed: 16740641]

9•. Mehta JP, Campian JL, Guardiola J, Cabrera JA, Weir EK, Eaton JW. Generation of oxidants by hypoxic human pulmonary and coronary smooth-muscle cells. Chest 2008;133(6):1410–1414. [PubMed: 18339777]A report suggesting that ROS production is reduced by hypoxia in cultured PASMC and coronary artery smooth muscle cells (CASMC). This is of special interest because the tissue was of human origin, the duration of hypoxia was ≥1hr, and specifically that the response of CASMC was qualitatively similar to that of PASMC, suggesting that differences in contractile response to hypoxia relate to downstream effectors rather than O_2 sensing. Notably, in PASMC the majority of ROS was mitochondrial in origin, whereas in CASMC only ~30% was mitochondrial. See also Ref 10

10. Wu W, Platoshyn O, Firth AL, Yuan JX. Hypoxia divergently regulates production of reactive oxygen species in human pulmonary and coronary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2007;293(4):L952–959. [PubMed: 17693484]

11•. Schach C, Xu M, Platoshyn O, Keller SH, Yuan JX. Thiol oxidation causes pulmonary vasodilation by activating K+ channels and inhibiting store-operated Ca2+ channels. Am J Physiol Lung Cell Mol Physiol 2007;292(3):L685–698. [PubMed: 17098807]This paper reports that the thiol oxidising agent diamide causes relaxation of PA rings both via activation of K^+ channels, as suggested by previous studies, but also via inhibition of SOCE. This provides indirect support for the Redox hypothesis, which suggests hypoxia causes the cytosol to become more reduced thus inhibiting K_V channels, but also implies that a more reduced environment might enhance SOCE, thought to be an important component of HPV

12. Wang QS, Zheng YM, Dong L, Ho YS, Guo Z, Wang YX. Role of mitochondrial reactive oxygen species in hypoxia-dependent increase in intracellular calcium in pulmonary artery myocytes. Free Radic Biol Med 2007;42(5):642–653. [PubMed: 17291988]

13••. Rathore R, Zheng YM, Niu CF, Liu QH, Korde A, Ho YS, Wang YX. Hypoxia activates NADPH oxidase to increase [ROS]i and [Ca2+]i through mitochondrial ROS-PKCε signaling axis in pulmonary artery smooth muscle cells. Free Radic Biol Med 2008;45(9):1223–1231. [PubMed:

18638544]In this intriguing study it was shown that hypoxia (~10 min) caused activation of NADPH oxidase (NOX) and translocation of $p47^{phox}$ in mouse PA but not mesenteric artery, and that inhibition of NOX or gene deletion of $p47^{phox}$ attenuated the hypoxia-induced elevation of ROS, $[Ca^{2+}]_i$ and force in PA. PKC inhibitors and PKCε gene deletion were used to identify a key role for PKCε, whereas use of mitochondrial inhibitors, genetic manipulation of glutathione peroxidase and exogenous peroxide provided evidence that the initial event was an increase in mitochondrial ROS generation, leading to activation of PKCε and hence NOX. This suggests that during acute hypoxia ROS derived from mitochondria activate NOX to produce more ROS, thus potentiating the elevation of $[Ca^{2+}]_i$ and contraction. Note that the time course of these experiments coupled with previous studies suggests that this phenomenon may only apply to phase 1 of HPV (see commentary on this paper, Ref 20).

14. Waypa GB, Guzy R, Mungai PT, Mack MM, Marks JD, Roe MW, Schumacker PT. Increases in mitochondrial reactive oxygen species trigger hypoxia-induced calcium responses in pulmonary artery smooth muscle cells. Circ Res 2006;99(9):970–978. [PubMed: 17008601]

15. Wardman P. Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic Biol Med 2007;43(7):995–1022. [PubMed: 17761297]

16•. Pourmahram GE, Snetkov VA, Knock GA, Shaifta Y, Drndarski S, Aaronson PI, Ward JPT. Constriction of pulmonary artery by peroxide: role of Ca(2+) release and PKC. Free Radic Biol Med 2008;45(10):1466–1476.A study performed in small PA of rat, showing that low concentrations of peroxide (30 M) cause sustained vasoconstriction and elevation of $[Ca^{2+}]_i$ due to release from ryanodine-sensitive Ca^{2+} stores. Notably, the response was unaffected by removal of extracellular Ca^{2+} and independent of Ca^{2+} entry. Intriguingly, the sustained constriction induced by peroxide involved Ca^{2+} sensitisation, but was independent of ROCK, or phosphorylation of MYPT1 or MLC_{20}. It was however abolished by inhibition of PKC and associated with translocation of PKCα, leading to the suggestion that peroxide activates a PKC-dependent actin-based mechanism in addition to mobilisation of ryanodine-sensitive Ca^{2+} stores.

17•. Lin MJ, Yang XR, Cao YN, Sham JS. Hydrogen peroxide-induced Ca2+ mobilization in pulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2007;292(6):L1598–1608. [PubMed: 17369291]Peroxide >10 μM was shown to cause an elevation in $[Ca^{2+}]_i$ in PASMC consisting of two components, the first of which was unaffected by removal of extracellular Ca^{2+} but was suppressed by ryanodine, thapsigargin and 2-aminoethyldiphenylborate, an inhibitor of IP_3 receptors. The second component, examined at 300 μM peroxide, was however dependent on Ca^{2+} entry via a pathway independent of voltage-gated Ca^{2+} channels, SOCE or receptor gated channels. Whilst the initial response to peroxide is consistent with other reports showing Ca^{2+} release from ryanodine sensitive stores and the ROS hypothesis, the relevance of the second component to HPV is as yet undefined.

18. Hodyc D, Snorek M, Brtnicky T, Herget J. SOD mimetic tempol inhibits hypoxic pulmonary vasoconstriction in rats independently on nitric oxide production. Exp Physiol. 2007

19••. Jernigan NL, Walker BR, Resta TC. Reactive oxygen species mediate RhoA/Rho kinase-induced Ca2+ sensitization in pulmonary vascular smooth muscle following chronic hypoxia. Am J Physiol Lung Cell Mol Physiol 2008;295(3):L515–529. [PubMed: 18621909]This study provides evidence that ROS-dependent activation of RhoA/ROCK signalling mediates enhanced Ca^{2+} sensitisation of endothelin-1 constriction of small, endothelium-disrupted, hypertensive PA isolated from chronically hypoxic rats ROS production is responsible for both increased basal and endothelin-1-stimulated, ET_A receptor-mediated activation of RhoA/ROCK and constriction of the hypertensive PA. Further investigation is required to determine the molecular source (NADPH oxidase, mitochondria, xanthine oxidase, etc) of the ROS, mechanistic basis of its increased production in hypoxic hypertension, and whether and how its production is modulated by endothelium.

20. Ward JP. A Twist in the Tail: Synergism between mitochondria and NADPH oxidase in the hypoxia-induced elevation of reactive oxygen species in pulmonary artery. Free Radical Biology and Medicine 2008;45(9):1220–1222. [PubMed: 18786634]

21•. Robertson TP, Mustard KJ, Lewis TH, Clark JH, Wyatt CN, Blanco EA, Peers C, Hardie DG, Evans AM. AMP-activated protein kinase and hypoxic pulmonary vasoconstriction. Eur J Pharmacol 2008;595(1–3):39–43. [PubMed: 18703047]This report provides further evidence in support of the AMPK hypothesis of O_2 sensing and HPV. Hypoxia caused increased phosphorylation of the

classical AMPK substrate acetyl CoA carboxylase, which was attenuated by the AMPK inhibitor compound C (40 μM); this strongly suggests that AMPK is activated by hypoxia. Compound C also selectively suppressed the sustained phase of HPV in isolated PA, without affecting phase 1. This study is consistent with activation of AMPK being instrumental to at least the sustained component of HPV, but may be limited due to the recently described non-selective actions of compound C (see Ref 22).

22. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P. The selectivity of protein kinase inhibitors: a further update. Biochem J 2007;408(3):297–315. [PubMed: 17850214]

23••. Cogolludo A, Moreno L, Frazziano G, Moral-Sanz J, Menendez C, Castaneda J, Gonzalez C, Villamor E, Perez-Vizcaino F. Activation of neutral sphingomyelinase is involved in acute hypoxic pulmonary vasoconstriction. Cardiovasc Res. 200910.1093/cvr/cvn349Shows that hypoxia causes increased synthesis of ceramide by neutral sphingomyelinase, in PA, with consequent activation of PKCξ and inhibition of K_V channels. Inhibition of neutral sphingomyelinase or PKCξ strongly suppressed hypoxia-induced inhibition of K_V currents in PASMC and HPV in isolated arteries and in vivo. This novel mechanism offers an alternative means by which hypoxia can cause inhibition of K_V channels. Interestingly, ceramide also activates ROCK, and exogenous ceramide evoked contriction which was suppressed by inhibition of ROCK.

24•. Platoshyn O, Yu Y, Ko EA, Remillard CV, Yuan JX. Heterogeneity of hypoxia-mediated decrease in IKv and increase in [Ca2+]cyt in pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2007;293(2):L402–416. [PubMed: 17526598]A careful study using combined single cell RT-PCR and patch clamp, showing heterogeneity between cultured PASMC isolated from the same artery in terms of $K_V1.5$ expression and the electrophysiological response to hypoxia. Notably, only ~46% of PASMC responded to hypoxia with inhibition of IK_V, and this was dependent on the level of expression of $K_V1.5$. In addition, hypoxia only elicited an elevation of $[Ca^{2+}]_i$ due to release from cyclopiazonic acid (CPA)-sensitive stores in ~34% of PASMC. The authors conclude that differences in not only $K_V1.5$ expression but also Ca^{2+} handling mechanisms may underlie this heterogeneity, and speculate that the subset of hypoxia-responsive PASMC in a PA segment can elicit uniform contraction by signalling to non-responsive PASMCs via gap junctions.

25•. Cha CY, Earm KH, Youm JB, Baek EB, Kim SJ, Earm YE. Electrophysiological modelling of pulmonary artery smooth muscle cells in the rabbits--special consideration to the generation of hypoxic pulmonary vasoconstriction. Prog Biophys Mol Biol 2008;96(1–3):399–420. [PubMed: 17915297]A mathematical model of PASMC was used to examine the contribution of various ionic currents to regulation of membrane potential. The key findings suggest that K_V and NSCC currents are the prime determinants of membrane potential in PASMC, but that hypoxic-inhibition of IK_V and IK_{Ca} is insufficient to cause the degree of depolarisation required for constriction, unless current through NSCC is increased 2.5 fold. This has potentially important ramifications for the mechanisms of Ca^{2+} entry during HPV, and suggests that a “two hit” model is required for depolarisation, as suggested by some previous studies.

26•. Firth AL, Yuill KH, Smirnov SV. Mitochondria dependent regulation of KV currents in rat pulmonary artery smooth muscle cells. AmJ Physiol Lung Cell MolPhysiol 2008;295(1):L61–70.The authors demonstrate an entirely novel mechanism that potentially links hypoxic-inhibition of mitochondrial function directly to K_V channels, in addition to any effects of ROS or redox state. They show that mitochondrial inhibitors acting at any point of the proximal ETC has a dual effect on IK_V, mediated by ATP and Mg^{2+}, such that at negative potentials IK_V was enhanced with a negative shift in K_V activation, whereas at positive potentials IK_V was suppressed. This suggests that when mitochondrial function is suppressed in hypoxia, the former mechanism would act against inhibition of K_V due to ROS or a more reduced redox state, and that an additional depolarising influence might be required for the latter to have a significant effect on membrane potential (see also Ref 25).

27••. Firth AL, Gordienko DV, Yuill KH, Smirnov SV. Cellular Localization of Mitochondria Contributes to Kv Channel Mediated Regulation of Cellular Excitability in the Pulmonary, but not Mesenteric Circulation. Am J Physiol Lung Cell Mol Physiol. 200810.1152/ajplung.90341.2008A follow up study to Ref. 26, showing that in freshly isolated PASMC, mitochondria and SR are much more closely approximated to the cell membrane than in smooth muscle cells from mesenteric artery, a

spatial arrangement that facilitates mitochondrial signalling to the PASMC membrane. This could represent a fundamental difference between PA and systemic arteries.

28•. Li XQ, Zheng YM, Rathore R, Ma J, Takeshima H, Wang YX. Genetic evidence for functional role of ryanodine receptor 1 in pulmonary artery smooth muscle cells. Pflugers Arch. 200810.1007/s00424-008-0556-8RyR1−/− and RyR1+/− mice were shown to exhibit suppression of spontaneous and caffeine-, IP_3- and neurotransmitter-induced Ca^{2+} release in PASMC and associated constriction of PA, and also of the elevation of $[Ca^{2+}]_i$ and contraction elicited by depolarisation with high $[K^+]$ in both Ca^{2+}-free and Ca^{2+}-containing solutions. Importantly, the hypoxia-induced elevation of $[Ca^{2+}]_i$ in PASMC was largely blocked in RyR−/− and RyR+/− mice. Although this study confirms the importance of ryanodine-sensitive stores and specifically RyR1 in HPV, it raises questions concerning the link between RyR1 and depolarisation.

29. Zheng YM, Wang QS, Liu QH, Rathore R, Yadav V, Wang YX. Heterogeneous gene expression and functional activity of ryanodine receptors in resistance and conduit pulmonary as well as mesenteric artery smooth muscle cells. J Vasc Res 2008;45(6):469–479. [PubMed: 18434746]

30••. Kinnear NP, Wyatt CN, Clark JH, Calcraft PJ, Fleischer S, Jeyakumar LH, Nixon GF, Evans AM. Lysosomes co-localize with ryanodine receptor subtype 3 to form a trigger zone for calcium signalling by NAADP in rat pulmonary arterial smooth muscle. Cell Calcium 2008;44(2):190–201. [PubMed: 18191199]NAADP has been shown to elicit localised Ca^{2+} release from a lysosome-related store in artery smooth muscle cells, which is amplified into Ca^{2+} waves by Ca^{2+}-induced Ca^{2+} release from RyR in SR Ca^{2+} stores. This study suggests that a population of lysosomes associates with RyR3-containing SR Ca^{2+} stores in the perinuclear region of PASMC, and forms a trigger zone for NAADP Ca^{2+} signalling. As RyR3 have been associated with the greater elevation in $[Ca^{2+}]_i$ to hypoxia in distal PA, this has potential implications for HPV.

31. Wilson HL, Dipp M, Thomas JM, Lad C, Galione A, Evans AM. ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase act as a redox sensor. a primary role for cyclic ADP-ribose in hypoxic pulmonary vasoconstriction. J Biol Chem 2001;276(14):11180–11188. [PubMed: 11116136]

32. Robertson TP, Hague D, Aaronson PI, Ward JP. Voltage-independent calcium entry in hypoxic pulmonary vasoconstriction of intrapulmonary arteries of the rat. J Physiol 2000;525(Pt 3):669–680. [PubMed: 10856120]

33•. Ng LC, Kyle BD, Lennox AR, Shen XM, Hatton WJ, Hume JR. Cell culture alters Ca2+ entry pathways activated by store-depletion or hypoxia in canine pulmonary arterial smooth muscle cells. Am J Physiol Cell Physiol 2008;294(1):C313–323. [PubMed: 17977940]This study shows that in canine freshly isolated PASMC, depletion of Ca^{2+} stores with cyclopiazonic acid (CPA) abolishes the Ca^{2+} transient induced by 5-HT but not that induced by caffeine, suggesting separate IP_3 and ryanodine sensitive stores. However, following cell culture these stores become reorganised into a single pool, and depletion of this pool by hypoxia or CPA subsequently activates SOCE, voltage-gated Ca^{2+} entry, and reverse-mode Na^+-Ca^{2+} exchange (NCX) which was not seen in fresh cells. The authors suggest that activation of NCX may be due to Na^+ entry via NSCC associated with SOCE. This report raises serious questions concerning the use of cultured PASMC for the study of Ca^{2+} mobilisation during hypoxia.

34•. Ng LC, Wilson SM, McAllister CE, Hume JR. Role of InsP3 and ryanodine receptors in the activation of capacitative Ca2+ entry by store depletion or hypoxia in canine pulmonary arterial smooth muscle cells. Br J Pharmacol 2007;152(1):101–111. [PubMed: 17592501]This group have previously reported that activation of SOCE in canine PASMC requires simultaneous depletion of separate IP_3- and ryanodine-sensitive Ca^{2+} stores. In this study they examined the role of RyR and IP_3R in store depletion- and hypoxia-induced SOCE. Following store depletion with caffeine and 5-HT, application of the IP_3R antagonists xestospongin or 2-APB abolished SOCE on reintroduction of external Ca^{2+}, whereas it was unaffected by inhibitors of RyR. Conversely, activation of SOCE by hypoxia was unaffected by xestopongin. This suggests that IP_3R play a role in SOCE per se rather than just the process of store depletion, but not in SOCE induced by hypoxia. This implies that store depletion and hypoxia may activate different SOCE pathways.

35. Becker S, Knock GA, Snetkov V, Ward JP, Aaronson PI. Role of capacitative Ca2+ entry but not Na+/Ca2+ exchange in hypoxic pulmonary vasoconstriction in rat intrapulmonary arteries. Novartis Found Symp 2006;272:259–268. [PubMed: 16686440]discussion 268–279

36. Becker S, Moir LM, Snetkov VA, Aaronson PI. Hypoxic pulmonary vasoconstriction in intact rat intrapulmonary arteries is not initiated by inhibition of Na+-Ca2+ exchange. Am J Physiol Lung Cell Mol Physiol 2007;293(4):L982–990. [PubMed: 17616643]

37•. Zhang S, Dong H, Rubin LJ, Yuan JX. Upregulation of Na+/Ca2+ exchanger contributes to the enhanced Ca2+ entry in pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension. Am J Physiol Cell Physiol 2007;292(6):C2297–2305. [PubMed: 17192285]Expression of the NCX1 isoform of the Na^+/Ca^{2+} exchanger proteins was upregulated in human hypertensive PASMC, and depletion of intracellular Ca^{2+} stores not only increased $[Ca^{2+}]_i$ due to SOCE but also stimulated Ca^{2+} entry via the reverse mode of Na^+/Ca^{2+} exchange. Thus, enhanced Ca^{2+} entry via the reverse mode of Na^+/Ca^{2+} exchange can contribute to the elevated [Ca^{2+} vasoconstriction $]_i$, which may be a trigger for pulmonary and stimulus for smooth muscle cell proliferation and migration in IPAH.

38. Hewavitharana T, Deng X, Soboloff J, Gill DL. Role of STIM and Orai proteins in the store-operated calcium signaling pathway. Cell Calcium 2007;42(2):173–182. [PubMed: 17602740]

39. Snetkov VA, Aaronson PI, Ward JP, Knock GA, Robertson TP. Capacitative calcium entry as a pulmonary specific vasoconstrictor mechanism in small muscular arteries of the rat. Br J Pharmacol 2003;140(1):97–106. [PubMed: 12967939]

40. Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S. STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. Nat Cell Biol 2007;9(6):636–645. [PubMed: 17486119]

41•. Lu W, Wang J, Shimoda LA, Sylvester JT. Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca2+ responses to hypoxia. Am J Physiol Lung Cell Mol Physiol 2008;295(1):L104–113. [PubMed: 18424621] Analysis of SOCE in PASMC derived from conduit and distal PA of rats showed that SOCE was greatest in the small PA that show the greatest response to hypoxia. Moreover, these distal PA exhibited the greatest expression of STIM1 and TRPC1, 6 and 4. Whilst these data do not demonstrate any causative link between SOCE and protein expression, they are consistent with the concept that STIM1 and TRPC containing channels underlie the SOCE associated with HPV.

42••. Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, Tigno DD, Thistlethwaite PA, Rubin LJ, Yuan JX. Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. Proc Natl Acad Sci U S A 2004;101(38):13861–13866. [PubMed: 15358862]The expression of TRPC3 and −6 was higher in lung tissue and PASMC from IPAH patients than in those from normotensive or secondary PH patients. Inhibition of TRPC6 expression attenuated the increased rate of proliferation of IPAH PASMC. Upregulated TRPC channel activity may serve as a Ca^{2+} entry pathway to elevate $[Ca^{2+}]_i$ and stimulate pulmonary vasoconstriction and vascular remodelling. In conjunction with Ref 37 and earlier publications by the Yuan lab, it appears increased $[Ca^{2+}]_i$ in IPAH PASMC can be due to combined effects of Ca^{2+} entry via voltage-gated Ca^{2+} channels, reverse Na^+/Ca^{2+} exchange, and TRPC-dependent SOCE.

43•. Keseru B, Barbosa-Sicard E, Popp R, Fisslthaler B, Dietrich A, Gudermann T, Hammock BD, Falck JR, Weissmann N, Busse R, Fleming I. Epoxyeicosatrienoic acids and the soluble epoxide hydrolase are determinants of pulmonary artery pressure and the acute hypoxic pulmonary vasoconstrictor response. FASEB J. 200810.1096/fj.08-112821The role of cytochrome P450 (CYP)-derived EETs in HPV was examined in perfused lungs of mice. Inhibition or gene deletion of soluble epoxide hydrolase (sEH; metabolises EETs) enhanced, whilst inhibition of CYP or an EETs antagonist diminished HPV. 11,12-EET increased PA pressure, and like hypoxia caused translocation of a TRPC6-V5 fusion protein to the membrane; the EETs antagonist diminished the translocation induced by hypoxia. TRPC6−/− mice did not respond to 11,12-EET or hypoxia. These results suggest that EETs promote translocation of TRPC6, and hence contribute to HPV. It should be noted that the duration of hypoxia in this study corresponds to phase 1 of HPV, which unlike the sustained phase 2 is abolished in TRPC6−/− mice (see Ref 2).

44. Nagaoka T, Morio Y, Casanova N, Bauer N, Gebb S, McMurtry I, Oka M. Rho/Rho kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats. Am J Physiol Lung Cell Mol Physiol 2004;287(4):L665–672. [PubMed: 12959926]

45. McNamara PJ, Murthy P, Kantores C, Teixeira L, Engelberts D, van Vliet T, Kavanagh BP, Jankov RP. Acute vasodilator effects of Rho-kinase inhibitors in neonatal rats with pulmonary hypertension

unresponsive to nitric oxide. Am J Physiol Lung Cell Mol Physiol 2008;294(2):L205–213. [PubMed: 18032699]

46•. Oka M, Fagan KA, Jones PL, McMurtry IF. Therapeutic potential of RhoA/Rho kinase inhibitors in pulmonary hypertension. Br J Pharmacol 2008;155(4):444–454. [PubMed: 18536743]Reviews a burgeoning body of evidence that RhoA/ROCK signalling is important in the pathogenesis of various experimental models of PH, including chronic hypoxia-, monocrotaline-, bleomycin-, shunt-, and vascular endothelial growth factor receptor inhibition plus chronic hypoxia-induced PH, and mild hypoxia-induced PH in neonatal fawn-hooded rats. Although information on RhoA/ROCK in human forms of PH is limited, it is also beginning to accumulate, e.g. see Ref 60.

47. Wang J, Weigand LA, Foxson J, Shimoda LA, Sylvester JT. Ca2+ signaling in hypoxic pulmonary vasoconstriction: effects of myosin light chain and rho kinase antagonists. Am J Physiol Lung Cell Mol Physiol 2007;293:L674–L685. [PubMed: 17575009]

48. Knock GA, Shaifta Y, Snetkov VA, Vowles B, Drndarski S, Ward JP, Aaronson PI. Interaction between src family kinases and rho-kinase in agonist-induced Ca2+-sensitization of rat pulmonary artery. Cardiovasc Res 2008;77(3):570–579. [PubMed: 18032393]

49. Badejo AM Jr, Dhaliwal JS, Casey DB, Gallen TB, Greco AJ, Kadowitz PJ. Analysis of pulmonary vasodilator responses to the Rho-kinase inhibitor fasudil in the anesthetized rat. Am J Physiol Lung Cell Mol Physiol 2008;295(5):L828–836. [PubMed: 18689606]

50•. Knock GA, Snetkov V, Shaifta Y, Connolly M, Drndarski S, Pourmahram GE, Aaronson PI, Ward JP. Superoxide induces Rho kinase mediated Ca2+ sensitisation in pulmonary artery. Free Radic Biol Med. 200810.1016/j.freeradbiomed.2008.11.015This report demonstrates that superoxide causes sustained, Ca^{2+} signal independent vasoconstriction of small distal PA, mediated by ROCK-dependent phosphorylation of MYPT1 and MLC_{20}, and implicates an upstream role for SFK. Superoxide also caused translocation of ROCK in PASMC. On the other hand, superoxide either had no effect or relaxed small systemic arteries. As the response in PA was inhibited by superoxide dismutase but not catalase, and peroxide did not activate ROCK (see also Ref 16), this strongly implies that superoxide and peroxide have independent effects in PA, which has implications for the ROS hypothesis of HPV, with which this study is otherwise consistent.

51. Homma N, Nagaoka T, Morio Y, Ota H, Gebb SA, Karoor V, McMurtry IF, Oka M. Endothelin-1 and serotonin are involved in activation of RhoA/Rho kinase signaling in the chronically hypoxic hypertensive rat pulmonary circulation. J Cardiovasc Pharmacol 2007;50(6):697–702. [PubMed: 18091588]

52. Khromov AS, Wang H, Choudhury N, McDuffie M, Herring BP, Nakamoto R, Owens GK, Somlyo AP, Somlyo AV. Smooth muscle of telokin-deficient mice exhibits increased sensitivity to Ca^{2+} and decreased cGMP-induced relaxation. Proc Natl Acad Sci U S A 2006;103(7):2440–2445. [PubMed: 16461919]

53•. Madden JA, Dantuma MW, Sorokina EA, Weihrauch D, Kleinman JG. Telokin Expression and the Effect of Hypoxia on Its Phosphorylation Status in Smooth Muscle Cells from Small and Large Pulmonary Arteries. Am J Physiol Lung Cell Mol Physiol 2008;294(6):L1166–1173. [PubMed: 18375742]Telokin, a target for protein kinase G, enhances myosin phosphatase activity in its phosphorylated form and promotes relaxation. Whereas telokin was not expressed in feline cerebral artery, in PA its expression varied inversely with diameter. Hypoxia caused dephosphorylation of telokin in small distal PA which exhibit HPV, thus presumably enhancing the response, but not in large PA which do not exhibit HPV. Whilst the means by which hypoxia modulates telokin phosphorylation is unclear, this study implies an entirely novel mechanism by which Ca^{2+} sensitivity may be regulated in HPV.

54••. Oka M, Homma N, Taraseviciene-Stewart L, Morris KG, Kraskauskas D, Burns N, Voelkel NF, McMurtry IF. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats. Circ Res 2007;100(6):923–929. [PubMed: 17332430]Demonstrates ROCK-mediated vasoconstriction is a major component of the high PVR in a rat model of severe PH that develops obstructive lesions in small precapillary PA. While ROCK-mediated vasoconstriction continues to be a major factor, the nadir to which right ventricular systolic pressure is reduced by acute ROCK inhibition increases over duration of PH and density of obstructive PA lesions.

55•. Broughton BR, Walker BR, Resta TC. Chronic hypoxia induces Rho kinase-dependent myogenic tone in small pulmonary arteries. Am J Physiol Lung Cell Mol Physiol 2008;294(4):L797–806.

[PubMed: 18263668]Assessed contribution of ROCK to increased basal tone and pressure-induced constriction in endothelium-disrupted small PA from control and chronically hypoxic rats. ROCK inhibition reduced basal tone in hypertensive PA but had no effect in control PA. Stepwise increases in intraluminal pressure caused constriction of the hypertensive PA that occurred independently of any change in $[Ca^{2+}]_i$ and was abolished by ROCK but not PKC inhibition. The mechanism by which chronic hypoxia augments the stretch-induced activation of ROCK is unknown, but this myogenicity likely contributes to the sustained ROCK-dependent vasoconstriction in hypoxic PH.

56•. Hirenallur SD, Haworth ST, Leming JT, Chang J, Hernandez G, Gordon JB, Rusch NJ. Upregulation of vascular calcium channels in neonatal piglets with hypoxia-induced pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2008;295(5):L915–924. [PubMed: 18776054]Both perfused hypertensive lungs and isolated PA from chronically hypoxic piglets demonstrated elevated Ca^{2+} channel-dependent vascular tone as reflected in the acute response to nifedipine. This was associated with an early and persistent upregulation of the pore-forming α_{1C}-subunit of the L-type Ca^{2+} channel in small hypertensive PA. A similar phenomenon may explain the effectiveness of Ca^{2+} channel blockers against PH in a subset of infants and children with PH.

57. Nagaoka T, Gebb SA, Karoor V, Homma N, Morris KG, McMurtry IF, Oka M. Involvement of RhoA/Rho kinase signaling in pulmonary hypertension of the fawn-hooded rat. J Appl Physiol 2006;100(3):996–1002. [PubMed: 16322374]

58. Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Thebaud B, Bonnet S, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir EK, et al. An abnormal mitochondrial-hypoxia inducible factor-1alpha-Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. Circulation 2006;113(22):2630–2641. [PubMed: 16735674]

59. Mair KM, MacLean MR, Morecroft I, Dempsie Y, Palmer TM. Novel interactions between the 5-HT transporter, 5-HT1B receptors and Rho kinase in vivo and in pulmonary fibroblasts. Br J Pharmacol 2008;155(4):606–616. [PubMed: 18695640]

60. Desbuards N, Antier D, Rochefort GY, Apfeldorfer CS, Schenck E, Hanton G, Hyvelin JM. Dexfenfluramine discontinuous treatment does not worsen hypoxia-induced pulmonary vascular remodeling but activates RhoA/ROCK pathway: Consequences on pulmonary hypertension. Eur J Pharmacol. 200810.1016/j.ejphar.2008.11.025

61••. Laumanns IP, Fink L, Wilhelm J, Wolff JC, Mitnacht-Kraus R, Graef-Hoechst S, Stein MM, Bohle RM, Klepetko W, Hoda MAR, Schermuly RT, et al. The Non-canonical WNT-pathway Is Operative in Idiopathic Pulmonary Arterial Hypertension. Am J Respir Cell Mol Biol. 200810.1165/rcmb.2008-0153OCThis study found upregulation in IPAH PA of several mediators of the canonical and non-canonical, the planar cell polarity, WNT pathways, the latter of which included wingless member 11, disheveled associated activator of morphogenesis-1, disheveled, Rac1, RhoA and ROCK. Immunohistochemical staining confirmed the enhanced expression of the planar cell polarity pathway mediators in both large and small PA. Functional studies are required to identify the contributions of the canonical and non-canonical WNT pathways to the pathogenesis of IPAH. At least two other reports of increased RhoA/ROCK activity in human hypertensive PA are currently under review.

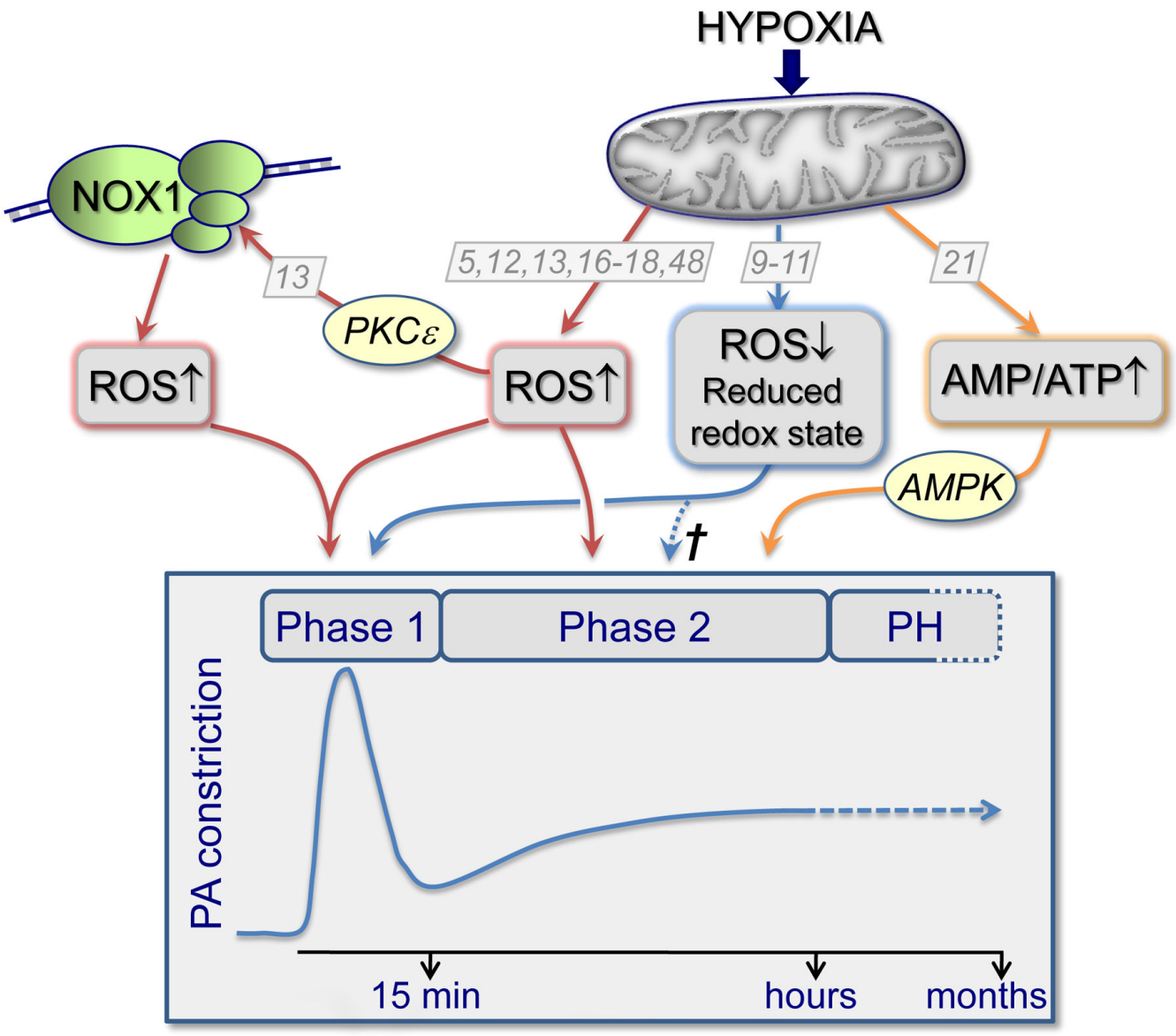

Figure 1. Initiation of HPV

Representation of the Redox, ROS and AMPK hypotheses and their potential signalling pathways lxinking the mitochondrial O_2 sensor to the initial and sustained components of HPV. The boxes highlight studies from the last 2 years that lend support to and/or suggest additional pathways for each hypothesis. †: as yet, no mechanisms have been implicated by which the Redox hypothesis could account for identified key components of phase 2 of HPV, notably Ca^{2+} release from ryanodine-sensitive stores and activation of ROCK (see text). AMPK, AMP-activated kinase; NOX1, NADPH oxidase; PH, chronic hypoxic pulmonary hypertension; ROS, reactive oxygen species.

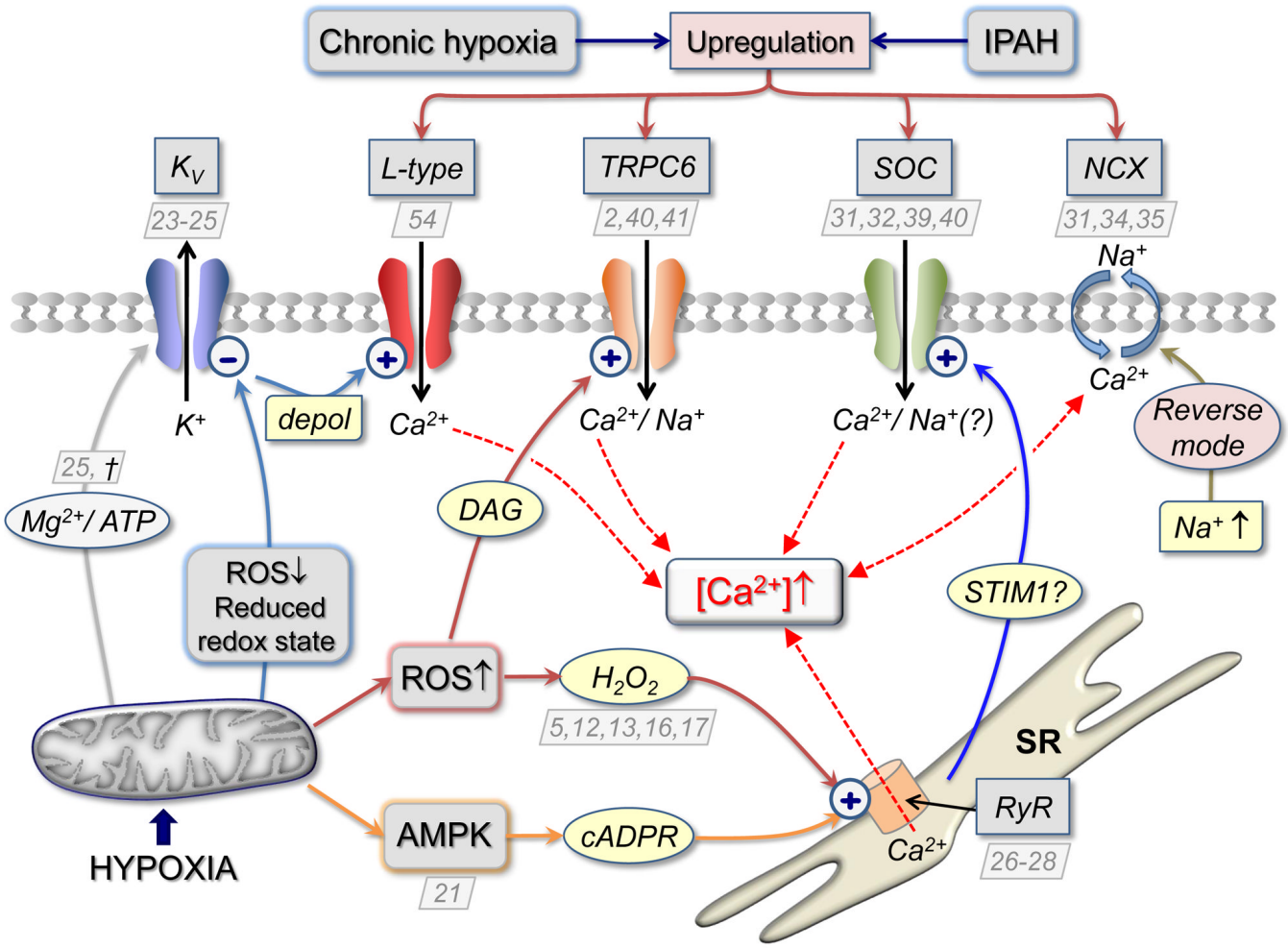

Figure 2. Ca^{2+} mobilisation in HPV

Mechanisms that have been implicated in the hypoxia-induced elevation of $[Ca^{2+}]_i$, and their potential signalling pathways. Note that some mechanisms are probably only applicable to one phase of HPV (see text). Not shown for clarity: possibility of functionally different types of SOC and Ca^{2+} store. Note that Na^+ entry via NSCC would also contribute to depolarisation. The boxes highlight studies from the last 2 years that address that pathway or channel. †: action of Mg^{2+} and ATP on K_V channels depends on membrane potential (see text). DAG, diacylglycerol; cADPR, cyclic ADP ribose; depol, depolarization; K_V, voltage-gated K^+ channels; L-type, voltage-gated Ca^{2+} channels; NCX, Na^+-Ca^{2+} exchanger; RyR, ryanodine receptors; SOC, store operated channels.

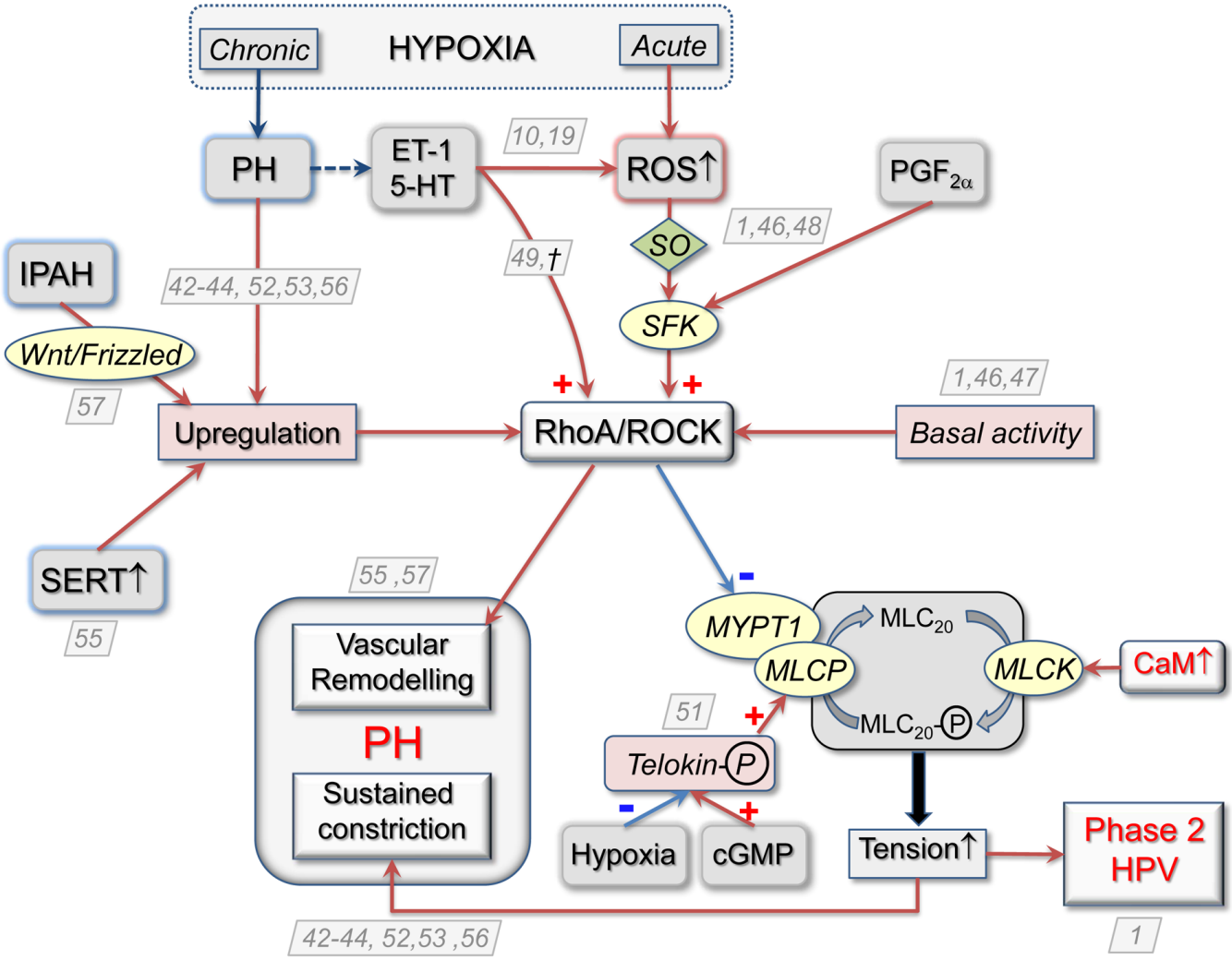

Figure 3. Ca^{2+} sensitisation and ROCK-mediated pathways in HPV and PH

Mechanisms that have been implicated in the activation and modulation of Ca^{2+} sensitisation pathways in HPV, and sustained vasoconstriction and vascular remodelling in PH, showing the central role of RhoA and ROCK. Not shown: the influence of ROS, RhoA/ROCK and PKC on the cytoskeleton, which could modulate Ca^{2+} mobilisation, actin-myosin interactions, and remodelling. The boxes highlight studies from the last 2 years that address that pathway. †: the pathways mediating activation of ROCK via 5-$HT_{1B/1D}$ receptors have not been established, but could include SFK. 5-HT, serotonin, 5-hydroxytryptamine; CaM, Ca^{2+}-calmodulin; ET-1, endothelin-1; Frizzled, Wnt receptor; IPAH, idiopathic pulmonary arterial hypertension; MP, myosin phosphatase; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SERT, serotonin transporter; SFK, src-family kinases; SO, superoxide.

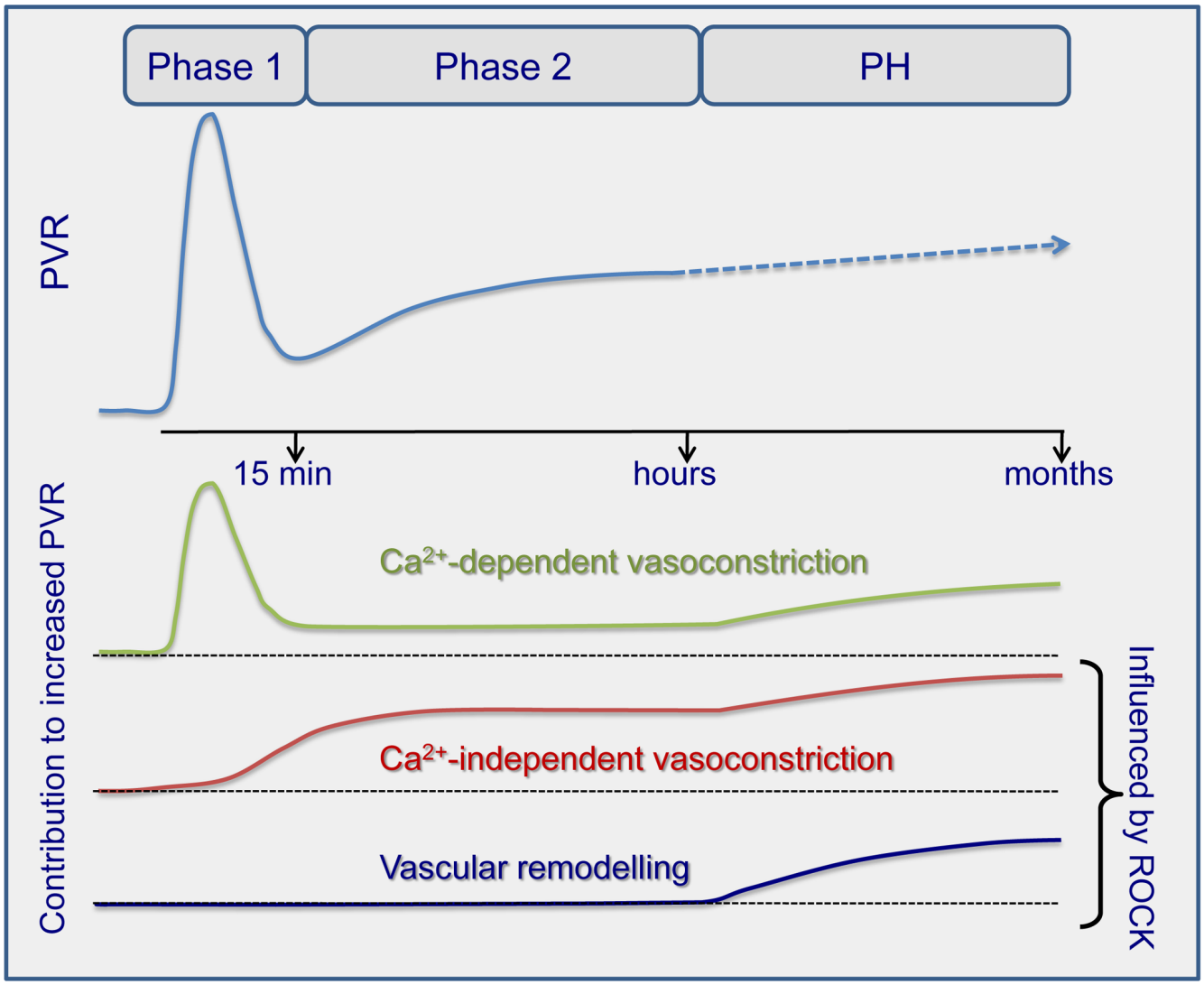

Figure 4.
Hypothetical diagram showing the contribution to the hypoxia-induced elevation in pulmonary vascular resistance (PVR) of Ca^{2+}-dependent and -independent vasoconstriction and vascular remodelling. Inhibitors of ROCK or its activation pathways may therefore be of benefit for PH, without significantly impairing acute ventilation-perfusion matching.