TRPC Channel Upregulation in Chronically Hypoxic Pulmonary Arteries

The HIF-1 Bandwagon Gathers Steam

Philip I. Aaronson

C hronic alveolar hypoxia causes structural and functional changes in pulmonary arteries (PAs) leading to increased pulmonary vascular resistance, secondary pulmonary hypertension (SPH), and, as a possible consequence, right heart failure. These changes are likely to result, at least in part, from the altered expression of ion channels in pulmonary arterial smooth muscle cells (PASMCs), and recent reports indicate that an increase in the expression of TRPC (canonical transient receptor potential) channels in these cells may play an important role. What has not been evident, however, is the mechanism by which chronic hypoxia (CH) enhances TRPC channel expression in these cells. In this issue of Circulation Research, Wang and colleagues1 now elegantly demonstrate that an increased expression of TRPC1 and TRPC6 in PASMCs during CH in mice and rats is mediated by the ubiquitous oxygen-sensitive transcription factor hypoxia-inducible factor 1 (HIF-1).

Effects of Chronic Hypoxia on Pulmonary Arteries: A Role for TRPC Channels

Prolonged exposure of the pulmonary vasculature to hypoxia evokes structural alterations including hypertrophy and hyperplasia of PASMCs leading to thickening of PA and neomuscularization of pulmonary arterioles. At the same time, pulmonary vascular reactivity is generally increased; basal tone rises, as does agonist-stimulated vasoconstriction, whereas endothelium-dependent dilation diminishes.2 Conversely, CH depresses PA constriction elicited by acute hypoxia (hypoxic pulmonary vasoconstriction).3

On a cellular level, CH causes PASMC membrane depolarization associated with a reduced expression and function of voltage-gated K⁺ channels.4,5 This has been proposed to contribute to PASMC proliferation by causing a rise in [K⁺], and inhibiting apoptosis.6 A rise in the basal [Ca²⁺], of PASMC has also been reported.7,8 This does not appear to be primarily caused by the opening of voltage-dependent (L-type) Ca²⁺ channels secondary to membrane depolarization, because nifedipine had little7 or no⁸ effect on [Ca²⁺], in PASMCs from chronically hypoxic rats. Similarly, treatment with Ca²⁺ channel antagonists has more often than not failed to reverse SPH or prevent its development in humans and animals.⁹¹¹

Although depolarization might theoretically raise [Ca²⁺], by promoting IP₃-mediated release of Ca²⁺ from intracellular stores,¹² it seems increasingly evident that it is an increased expression of channels incorporating TRPC1 and TRPC6 proteins which is largely responsible for raising PASMC [Ca²⁺], during CH, and that this contributes to the pulmonary vascular remodelling and increased reactivity which ensue. A burgeoning literature has established that numerous TRP protein isoforms, mainly of the TRPC family, are expressed in vascular smooth muscle, and that they are involved in non–voltage-gated Ca²⁺ influx pathways than on L-type Ca²⁺ channels, whereas systemic arteries of similar diameter demonstrate the opposite dependency.¹⁴ Expression of TRPC1 and TRPC6 has been consistently observed in PASMCs, with expression of TRPC3 and TRPC4 also variably detected.⁷¹⁵⁻¹⁸

There is extensive evidence that cell cycle progression is Ca²⁺-dependent, as are the activities of multiple transcription factors.¹⁹ Yuan and colleagues demonstrated that PASMC proliferation was abolished when Ca²⁺ influx and release from the sarcoplasmic reticulum were prevented. TRPC1 was shown to play a crucial role in supplying Ca²⁺ for the growth of these cells, because their proliferation was associated with its increased expression, as well as a rise in both store-operated Ca²⁺ entry (SOCE) and in basal [Ca²⁺]. Moreover, knockdown of TRPC1 expression strongly suppressed PASMC proliferation and SOCE.¹⁵¹⁷ Subsequently, Lin and coworkers² described upregulation of both SOCE and OAG-stimulated (ie, receptor-activated) Ca²⁺ influx in PASMCs from chronically hypoxic rats. Experiments using La³⁺, which differentially blocked these two pathways, indicated that it was SOCE which was mainly responsible for the elevated [Ca²⁺], and basal tone in PA from hypoxic animals. CH also strongly enhanced the mRNA/protein expression of TRPC1 and TRPC6 (but not TRPC3). siRNA knockdown of TRPC1 and TRPC6 selectively suppressed SOCE and OAG-induced Ca²⁺ influx, respectively. The reports indicate that TRPC1 upregulation probably contributes to the increased basal pulmonary tone and hyperplasia/hypertrophy of the

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However, as O₂ levels fall, hydroxylation decreases accelerating its degradation and also causing functional inhibition on key proline and asparaginine residues, of two subunits, one of which, HIF-1α/H11002 is constitutively expressed. However the stability of the other subunit, HIF-1β/H9251, is regulated by cellular O₂ levels, as is its binding to translational coactivators. Under normoxic conditions, HIF-1α is hydroxylated by specific enzymes in an O₂-dependent manner on key proline and asparaginine residues, accelerating its degradation and also causing functional inhibition. However, as O₂ levels fall, hydroxylation decreases and the concentration of functional HIF-1 dimer rises. This allows it to promote the transcription of genes for numerous proteins, including erythropoietin, VEGF, and multiple glycolytic enzymes, which are crucial for adaptation to hypoxia.20,21

A role for HIF-1 in mediating the pulmonary responses to CH was first demonstrated by Yu et al.,22 who showed that the development of right ventricular hypertrophy, vascular remodelling, and SPH was delayed in Hif1a−/− mice compared with Hif1a+/+ mice. Similarly, PASMC hypertrophy, membrane depolarization, and K⁺ current attenuation elicited by CH were markedly reduced in the HIF-1α heterozygotes, compared with controls.23

The study by Wang et al. now provides compelling evidence that the developing stories of HIF-1 and TRPC channels in the pulmonary vascular response to CH form part of the same narrative. The authors first confirm previous observations of elevated [Ca²⁺], and SOCE in transiently-cultured PASMCs from rats made hypoxic for 21 days. They then show that in vivo chronic hypoxia in both rats and control mice results in an upregulation of the expression of protein and mRNA for TRPC1 and TRPC6, but not TRPC4. Crucially, they demonstrate that the increases in basal [Ca²⁺], and TRPC1/6 expression are absent in HIF1a−/− mice. Accordingly, overexpression of a constitutively active form of HIF-1α also increases the expression of TRPC1/6, but not TRPC4.

A key unresolved issue relates to the extent to which an increased expression of TRPC channels in pulmonary smooth muscle actually contributes to the overall sequence of events leading to secondary pulmonary hypertension. This question will only be answered when the in vivo effects of molecular or pharmacological ablation of TRPC1/6 signaling during CH are assessed. However, regardless of the centrality of this particular effect of HIF-1 to the pathogenesis of SPH, a clear implication of a series of studies from the Johns Hopkins group and its collaborators is that HIF-1 is probably responsible for multiple CH-induced alterations in the pulmonary vasculature, including the downregulation of K⁺ channels (Figure). Of particular importance in CH is the RhoA/Rho-kinase system, now increasingly seen as a central player in SPH.11 CH has been shown to upregulate the expression of Rho-kinase but not RhoA in PA.24 Although HIF-1 inversely downregulates Rho-kinase and increases RhoA expression, at least in fibroblasts,25 its possible role in controlling the expression of RhoA/Rho-kinase in the PA during CH remains unknown, and now becomes of great interest.

A variety of drugs are available which decrease the activity of HIF-1, although as yet no direct and specific inhibitors of HIF-1 have been described.21 Interest in the therapeutic targeting of HIF-1 is presently focused on suppressing its involvement in multiple cancers, however examination of the effects of pharmacological inhibitors of HIF-1 on the development of hypoxia-induced pulmonary hypertension in animal models would now also seem to be warranted.

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**Disclosures**

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**References**


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