Nitric Oxide–Mediated Zinc Release
A New (Modulatory) Pathway in Hypoxic Pulmonary Vasoconstriction

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Hypoxic pulmonary vasoconstriction (HPV) is an essential mechanism in the lung that redistributes pulmonary blood flow from areas of low oxygen tension to areas of high oxygen availability. This mechanism thus optimizes pulmonary gas exchange. Impairment of HPV can result in severe hypoxemia. However, under conditions of global alveolar hypoxia, it becomes grossly ineffective and acute pulmonary hypertension occurs because of the overall contraction of the resistance vessels of the lung. Moreover, permanent activation of hypoxic vasoconstriction may contribute to the structural remodeling process of the pulmonary vasculature in chronic hypoxia.

Despite the recognition of the importance of hypoxic vasoconstriction for pulmonary gas exchange by von Euler and Liljestrand in 1946, the underlying oxygen sensing and signal transduction processes have yet to be fully elucidated. Presently, mitochondria and NAD(P)H oxidases are proposed as possible oxygen sensors. However, there is great controversy as to the role of reactive oxygen species, i.e., whether they are up- or downregulated in hypoxia, mediating HPV. The original redox hypothesis assumes a decrease in reactive oxygen species (ROS), shifting the cellular redox state toward a more reduced state and resulting in the inhibition of K+ channels in pulmonary arterial smooth muscle cells. In contrast, there is also substantial evidence that an increase in ROS production during hypoxia triggers intracellular calcium release and thus the hypoxic vasoconstrictor response.

It is clearly evident that HPV is ultimately induced by an increase in intracellular calcium, and it is generally accepted that L-type calcium channels and K+ channels are essential components of the underlying signal transduction process. The AMP-activated protein kinase/cADP-ribose pathway and cytochrome P450 enzymes have also been suggested to contribute to the regulation of HPV, and recently, TRPC6 has been identified as an essential component, at least in mice.

NO is an important regulator of vascular tone in general, and there is manifold evidence that NO specifically interferes with HPV. It has repeatedly been shown that pulmonary NO release is reduced under hypoxia, and this reduction has been attributed to the lack of oxygen as a substrate for NO synthases. NO stimulates the soluble guanylate cyclase, which produces vasodilatory cGMP. Thus, a reduction in NO levels, caused by a reduced oxygen partial pressure, has been thought to contribute to HPV by a decreased activation of this vasodilatory pathway. In fact, the addition of NO to the pulmonary circulation resulted in specific suppression and inhibition of NO synthases amplified hypoxic pulmonary vasoconstriction.

Within this context, Bernal et al. describe in this issue of Circulation Research a new, NO-induced vasoconstrictor pathway for the pulmonary circulation via an interaction with metallothionein (MT) and provide evidence that this pathway is involved in the regulation of hypoxic vasoconstriction. MT is a metal-binding, zinc-storing protein. It has been suggested that NO via thiolate-nitrosation induces an intracellular conformational change of MT, resulting in zinc release from this protein. The data from Bernal et al. now substantiate the conclusion that (1) MT is a critical target for NO; (2) hypoxia induces an increase in NO in pulmonary arterial endothelial cells; and (3) the hypoxia-mediated increase in NO via nitrosation of MT results in a release of zinc from MT, which may, via a protein kinase C, induce pulmonary vasoconstriction (Figure). With regard to HPV, this signaling cascade is attributed to intraacinar endothelial cells, because the authors demonstrate that intraacinar vessels (<40 mm, without [a full] smooth muscle cell layer), as well as isolated pulmonary arterial endothelial cells, reversibly contract in response to hypoxia. The increase in endothelial NO was quantified by means of fluorescence resonance energy transfer sensors, and zinc release was detected by a fluorescent probe. Experiments in MT-deficient mice revealed a \( \approx 80\% \) reduction in HPV, which was specific for the hypoxia-induced vasoconstriction, because vasoconstriction induced by the thromboxane mimetic U46619 was not altered in MT\(^{-/-}\) mice compared with wild-type mice. With this study, Bernal et al. consequently extend the previous work of their laboratory with regard to NO-mediated zinc release to include a functional role in the pulmonary circulation, along with the previous observation of NO-mediated zinc trafficking by Spahl et al. The present report, thus, addresses a hitherto unrecognized pathway for the pulmonary circulation and challenges previous views about (the role of NO in) HPV but also raises important questions.
Is NO Increased or Decreased in the Pulmonary Circulation During Acute Hypoxia?

Although some controversy does exist, numerous investigations have reported a decrease in lung NO production/release under acute hypoxia (hypoxic periods of seconds to minutes), with this lack of NO contributing to HPV via a loss of vasodilatory capacity. Teleologically, such mechanisms evidently make sense, because the loss of NO would occur in hypoxic regions of the lung. For the pulmonary circulation only Hampel et al previously reported an increase of NO in pulmonary arterial endothelial cells, which has now been confirmed by Bernal et al for intracellular NO in the intact lung by an elegant methodology. It is suggested that this increase is caused by an activation of NO synthases stimulated by an increase in intracellular Ca\(^{2+}\) concentration. Resolving the mechanism of the proposed hypoxia-induced increase in lung NO synthesis requires further experimentation, and the discrepancy in this field with regard to the question of whether NO is decreased or increased in hypoxia may be caused by different contributions of different pulmonary cell types (endothelial, alveolar epithelial cells, airway epithelial cells, etc), as well as the techniques used for detection of NO generation. For example, quantification of exhaled and intravascularly released NO in isolated blood-free perfused lungs, which has exhibited decreased NO release during hypoxia, may differ in this regard with measurement in the presence of erythrocytes (hemoglobin, which is an NO scavenger) and with measurement of intracellularly detectable NO in a distinct compartment, as was performed by Bernal et al. Even NO release from alveolar epithelial cells can affect the strength of HPV by diffusion to the effector cells.

What Is the Contribution of Endothelial Cells/Nonmuscularized Intraacinar Vessels to the Physiology of HPV?

A variety of studies have tried to identify the site and cell types relevant for HPV. The early suggestion that the oxygen sensor and effector is located in different cells is complicated by the fact that HPV may consist of different phases, ie, (1) a very early response occurring within seconds and lasting \(\approx 30\) minutes and (2) a second phase of sustained vasoconstriiction, developing over hours. Moreover, (3) the pulmonary vasoconstrictor response to hypoxia, in a third phase lasting days to weeks, may contribute to the vascular remodeling process as the morphological correlate to chronic hypoxia-induced pulmonary hypertension. Although the existence of such phases may differ between different investigations/models, subsequent investigations have shown that the pathway specific for the very acute response seems to be mostly independent from the endothelium, although it can be modified by NO. For acute HPV (the phase that was investigated by Bernal et al), the most reasonable explanatory concept is that the oxygen-sensing and effector cells are smooth muscle cells located in the more or less fully muscularized precapillary vessels that distribute blood to the individual acini. This suggestion is based on the fact that these cells depolarize on hypoxia, increase their intracellular Ca\(^{2+}\), and contract. This concept suggests that each individual acinus as a whole is the smallest regulatory unit of the hypoxia-induced vasoconstrictor response. However, in 1997 Hillier et al showed that intraacinar vessels contract to hypoxia, and this observation now has been supported by the study of Bernal et al. In this regard, the question arises as to how the distal contraction of (nearly) nonmuscularized, intraacinar vessels contributes to physiologically relevant HPV. A sole regulation by endothelial cell contraction may entail the risk of lung edema formation, because these tiny structures are not capable of resisting high pulmonary pres-
sures. In addition, such a risk is potentiated if these cells contract, which may increase paracellular gaps.\textsuperscript{19} This discrepancy may be resolved by the speculation that 2 sites of HPV exist, the first, at the level of the muscularized precapillary acinar-feeding vessels, which are capable of resisting high pulmonary artery pressure; and a second, downstream of this area, regulated by endothelial cell contraction and responsible for an intracinar fine-tuning of ventilation perfusion distribution. Such a concept would protect intracinar areas against higher pressures by hypoxic constriction of the upstream resistance vessels. This suggestion may be addressed in future investigations, and the results/findings could very well vary between different species, speculatively attributable to the presence or the absence of the pores of Kohn, which allow interalveolar gas distribution. In this context, the question arises as to whether the mechanism of NO-induced vasoconstriction proposed by Bernal et al\textsuperscript{12} is also relevant for smooth muscle cells of the precapillary pulmonary vasculature.

**What Is the Proportion Between the NO-Induced Vasoconstrictor Pathway and the “Classic” NO-Induced Vasodilatory Pathway, and What Is Their Relevance for the Regulation of HPV?**

As stated above, there are multiple lines of evidence for the regulation of pulmonary vascular tone in general, and especially with regard to HPV, by the classical NO/guanylate cyclase/cGMP pathway (Figure). The vascular contraction on hypoxia is magnified by pharmacological NO inhibition, as well as in endothelial NO synthase–deficient mice.\textsuperscript{8–10,20} It has been suggested that the classical vasodilatory pathway is more efficient during high vascular tension compared with relaxed vessels and thus counterbalances an excessive vasoconstrictive profile.\textsuperscript{8} This issue is, however, complicated by the fact that Voelkel et al, in contrast to the notion of NO-mediated vasodilation, demonstrated that NO could also induce pulmonary vasoconstriction in the presence of hemolysate.\textsuperscript{21}

With regard to the study of Bernal et al,\textsuperscript{12} the question arises to what extent a MT/zinc–mediated vasoconstrictor effect is relevant for the in vivo situation and to what degree it competes with the NO-mediated vasodilatory pathway (Figure). The fact that HPV was blunted by \textasciitilde80\% in MT\textsuperscript{−/−} knockout mice suggests a major contribution of this pathway. However, in contrast zinc chelation, only reduced HPV during the blockade of the NO-mediated vasodilatory pathway.\textsuperscript{12} Moreover, because it was previously shown that hypoxic vasoconstriction can still be elicited (and even be increased) by total inhibition of lung NO synthesis, the MT/zinc pathway may also be triggered by NO-independent activity.\textsuperscript{8–10}

**What Is the Contribution of ROS and the ROS-NO Interaction to the MT/Zinc Pathway?**

Which is the exact downstream pathway of zinc release by MT? With regard to the present discussion of the contribution of superoxide to the regulation of HPV, it would be of interest to clarify the effects of altered ROS levels on NO availability and the MT/zinc pathway. Moreover, addressing the exact molecular mechanisms of the NO/MT/zinc pathway, eg, the identification of the targets of zinc release in the lung, may allow to specifically interfere with the new exciting pathway proposed by Bernal et al.\textsuperscript{12}

Although experiments with total blockade of NO synthesis have shown that acute HPV can still be elicited in the absence of any NO synthesis, the role of NO in modulating and, thus, tuning the pulmonary vasoconstrictor response in hypoxia to the physiological needs in vivo should not be underestimated. This is the case not only for the classic NO/guanylate cyclase/cGMP pathway but also for the new NO/MT/zinc pathway described by Bernal et al\textsuperscript{12} in this issue of Circulation Research, because the NO-dependent modulation of HPV has been shown to be specific for acute hypoxia-induced increase in vascular tension in comparison with non–hypoxia-induced vasoconstrictive stimuli.\textsuperscript{12}

Going beyond HPV, the physiological relevance of the proposed NO/MT/zinc–mediated vasoconstrictor pathway will have to be taken into account for pulmonary vasoregulation in general.

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**References**


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