Vascular Remodeling Versus Vasoconstriction in Chronic Hypoxic Pulmonary Hypertension
A Time for Reappraisal?

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Chronic or sustained pulmonary hypertension is a complication of residence at high altitudes and chronic lung diseases such as chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis, asthma, and sleep apnea. Alveolar hypoxia is an important (though probably not exclusive) contributor to the pulmonary hypertension observed in these conditions. Further, it is widely accepted that secondary hypoxic pulmonary hypertension is strongly associated with increased morbidity and reduced survival. These facts have led to intense research efforts to identify the underlying mechanisms contributing to this condition, with the ultimate goal of identifying and developing novel therapeutic interventions. This work has relied heavily on the use of animal models, and one of the most commonly used is exposure of rats to chronic-hypoxic conditions by nitrogen dilution or hypobaria. Observations, predominately in this model, have lead to the longstanding and widely accepted theory that chronic hypoxic pulmonary hypertension results from a combination of sustained vasoconstriction and vascular remodeling. It is generally believed that the contribution of vasoconstriction is greatest early in the disease process and that structural remodeling of the pulmonary vascular bed becomes progressively more important over time. That structural change is an important determinant of increased resistance and pressure in chronic pulmonary hypertension is supported by observations that over time of exposure to hypoxia, acute reexposure to normal or even high levels of inspired oxygen becomes progressively less effective in reducing the pulmonary arterial pressure. This lack of responsiveness to oxygen, or even to other pulmonary vasodilators such as Ca \(^{2+}\) channel blockers, has led to the concept that chronic hypoxic pulmonary hypertension is associated with a “fixed” structural component responsible for the increased pulmonary vascular resistance. The structural changes thought to contribute to the increased vascular resistance have been broadly characterized into 2 processes: (1) inward remodeling of the pulmonary artery wall and (2) a reduction in the total number of small peripheral pulmonary arteries (a process referred to as rarefaction or pruning of the pulmonary vasculature).

The work presented by Hyvel et al challenges, at least in certain ways, both of these concepts. Pulmonary vascular remodeling refers to a process that causes thickening of the arterial wall and is thought to increase resistance by physical encroachment of the lumen of small peripheral pulmonary arteries and arterioles. Because intimal thickening is not usually observed in hypoxic pulmonary hypertension, this reduction in luminal area is believed to be attributable largely to constrictive medial and adventitial thickening. However, using different lung preparation techniques than have been used in most other hypoxic studies (ie, the pulmonary vasculature was “maximally” vasodilated by perfusion with calcium-free plus EGTA physiological saline solution before the lungs were fixed by infusing paraformaldehyde into the trachea and pulmonary artery under “no flow” conditions at a defined transmural distending pressure that was constant at all points in the vasculature), the authors in this (and a previous) study have demonstrated that although some medial thickening clearly takes place in response to chronic hypoxia, there is, at least in this rat model, no reduction in the luminal area in vessels between 30 and 200 \(\mu\)m in diameter. Interestingly, this modest hypoxia-induced pulmonary vascular structural remodeling and absence of luminal narrowing in rats was previously reported by van Suylen et al, who also induced pulmonary vasodilation before lung fixation to abate any potential contribution of vasoconstriction to the observed pathologic changes (see Figure).

It is important to note that there are instances in the pulmonary circulation where remodeling (medial and adventitial thickening) of pulmonary resistance vessels occurs in the absence of significant pulmonary hypertension. For instance, in chronically-infected rat lungs pulmonary hypertension did not occur and right ventricular hypertrophy was not observed despite the appearance of thickened pulmonary vessel walls. Similar observations have been made in humans with chronic-obstructive pulmonary disease where marked thickening of the walls of the pulmonary arteries have been observed in the absence of pulmonary hypertension. One possible explanation for these findings is that thickening occurred in an outward direction such that it did not reduce the vascular lumen. This type of outward remodeling, called compensatory enlargement, has been well described in the systemic circulation. In fact, a dissociation between remodeling and pulmonary hypertension has been described. For instance, treatment of chronically hypoxic rats with angiotensin-converting enzyme inhibitors prevented pulmo-
nary artery medial thickening but not the pulmonary hypertension or right-ventricular hypertrophy.\textsuperscript{5,11} Thus, we need to consider the possibility that under certain circumstances remodeling of the pulmonary vasculature does not cause luminal narrowing and increased vascular resistance, and chronic pulmonary hypertension can develop in the absence of inward or maladaptive vascular remodeling.

It has been commonly accepted that the second major structural alteration caused by chronic hypoxia is loss of small peripheral pulmonary arteries, which increases resistance by reducing parallel vascular pathways.\textsuperscript{12–17} Traditionally, this rarefaction has been detected in barium-gelatin infused lungs as a reduction in the ratio of number of barium-filled blood vessels to number of alveoli in the intracinar (gas exchange) region of the lung. However, using the techniques of quantitative stereology combined with confocal microscopy the McLaughlin group presents evidence for hypoxia-induced angiogenesis in the pulmonary circulation.\textsuperscript{3,4} This technique allows statistical inferences about the 3D structural parameters of objects based on 2D information such as that provided by histologic images, and has made it possible to measure the total length of intracinar resistance vessels in the lung along with the total capillary surface area (parameters which have not traditionally been measured in most previous studies evaluating hypoxic pulmonary hypertension). Using these techniques, and also examining the pulmonary venules and gas exchange capillaries within the alveolar walls, areas again not usually examined in previous studies, the investigators have demonstrated that with chronic hypoxia the total combined length of the intracinar arteries and venules increases rather than decreases. Stereologic examination also revealed an increase in total capillary lumen volume, total endothelial cell surface area, and total endothelial cell number. Whether these changes are “angiogenic” in the conventional sense is not clear, because absolute increases in vessel density have not been firmly established. However, these observations are certainly in contrast to the majority of previous reports and challenge the concept that the mature pulmonary circulation does not undergo new growth.

There is previous experimental support for the idea that chronic hypoxia does not reduce pulmonary blood vessel number. Several studies of pulmonary hypertension in the rat have reported that the number of small pulmonary blood vessels is unaltered after chronic hypoxia\textsuperscript{18–22} or chronic inflammatory (monocrotaline-induced) lung disease.\textsuperscript{18} There are also other instances in which angiogenesis of the pulmonary circulation has been described. In adult dogs after pneumonectomy there is growth of new alveolar septa and alveolar capillaries in the residual left lung.\textsuperscript{23} Schraufnagel et al have reported evidence of pulmonary angiogenesis in the rat lung after induction of biliary cirrhosis.\textsuperscript{24} Schraufnagel has also suggested there is new vessel formation in the lungs of a patient with pulmonary venoocclusive disease.\textsuperscript{25} At least some metastatic tumors in the lung receive their blood supply either exclusively or predominately from the pulmonary circulation, again suggesting the ability of the pulmonary circulation to undergo an angiogenic response.\textsuperscript{26} In further support of the idea that pulmonary angiogenesis may occur in response to chronic hypoxia is the recent report from Pascaud et al, who found that angiostatin, an inhibitor of angiogenesis, aggravated pulmonary hypertension in the hypoxic lung.\textsuperscript{27} In addition, Beppu and colleagues have demonstrated hypoxia-induced capillary angiogenesis in the adult mouse lung.\textsuperscript{28} Collectively, these observations certainly raise the possibility that angiogenesis can, and does, occur in the mature pulmonary circulation in response to hypoxia and other pathophysiologic stimuli. This is not necessarily in conflict with evidence that the lung bronchial circulation may undergo even greater angiogenesis in response to similar stimuli.\textsuperscript{29}

The functional effects of these changes in capillary structure in the hypoxic lung are not entirely clear. There appears to be an increase in the total membrane diffusing capacity in chronic hypoxia, which could be attributable partly to the observed increase in total capillary volume. In addition, the increased capillary length caused by chronic hypoxia could prolong the time red blood cells spend in the alveolar capillaries at any given cardiac output, allowing more time for oxygen to equilibrate between alveolar gas and blood. However, whether any of these adaptations are real or pertinent to human hypoxic pulmonary hypertension is entirely unclear.

Caution must be exercised in interpreting the results regarding pulmonary vascular remodeling and angiogenesis in the rat model. There is great variation in the magnitude of the pulmonary hypertensive response to chronic hypoxia in different species, as well as in individuals within a species. Age and sex also influence the response to stimuli causing pulmonary hypertension, including hypoxia. We have demonstrated, using techniques in which the possibility of vasoconstriction contributing to reduction of vascular luminal area is largely eliminated, that chronic hypoxic exposure in the neonatal calf leads to substantial decreases in the cross-sectional area of the lumen of small pulmonary arteries,\textsuperscript{30} suggesting that at least under some circumstances hypoxia can indeed lead to inward remodeling and structural encroachment on the vascular lumen. In this neonatal calf model, the proliferative response observed in both the media and adventitia far exceeds that which has been reported in the rat. Thus, in the presence of excessive vascular cell proliferation and extremely high pressures there may be instances where structural luminal narrowing is induced by hypoxia. Whether or not the high vascular resistance and pressure associated with this narrowing in the calf is susceptible to marked acute reversal by Rho kinase inhibitors as has been reported in the hypoxic rat model\textsuperscript{3,31,32} remains open to question.

The current work and that of Nagaoka et al provide strong functional support for the morphological evidence discussed above that there is little, if any, inward structural remodeling and no significant loss of small pulmonary arteries in chronically hypoxic rats.\textsuperscript{3,31} Thus, both groups show that acute administration of a Rho kinase inhibitor nearly normalizes the increased pulmonary vascular resistance in both intact rats and isolated perfused lungs. The most likely explanation is that the increased pulmonary vascular resistance and pulmonary hypertension induced in adult male rats by up to 4 weeks of hypoxic exposure is attributable to sustained Rho kinase-
mediated pulmonary vasconstriction (Figure). The exact mechanisms of activation of Rho kinase in hypoxic rat lungs remain to be defined; Hyvel et al did not find activation of RhoA in whole lung tissue but Jernigan et al measured increased GTP-RhoA in hypertensive intrapulmonary arteries.36,37 Nagaoka et al have presented preliminary evidence that both endothelin-1 and serotonin may be involved in the sustained activation of RhoA/Rho kinase signaling in hypoxia-induced hypertensive rat pulmonary arteries.34

There is now considerable evidence that activation of the small GTPase RhoA and its downstream effector Rho kinase, RhoA/Rho kinase signaling, is involved in regulation of numerous cellular responses, including actin polymerization, gene transcription, differentiation, growth, migration, and contraction.35 Rho kinase causes inactivation of myosin light-chain phosphatase leading to increased myosin light-chain phosphorylation and thus increased smooth-muscle contraction.36,37 This signaling pathway is particularly important in mediating sustained vasoconstriction by increasing the Ca2+/sensitivity of smooth muscle cell contraction, and its activation has been implicated in the pathogenesis of several systemic vascular diseases.36,37 The current report indicates that the current report by Hyvel et al adds to a growing body of evidence that RhoA/Rho kinase signaling is also important in the pathogenesis of pulmonary hypertension, at least in rodents.31–33,38,39

As Fagan et al observed in mice, Hyvel et al show in rats that chronic treatment with a Rho kinase inhibitor attenuates development of hypoxic pulmonary hypertension.3,39 Prevention and reversal of rat monocrotaline-induced pulmonary hypertension by Rho kinase inhibition have also been reported.38

The current report indicates RhoA/Rho kinase signaling is also possibly involved in the hypoxia-induced pulmonary capillary angiogenesis the McLoughlin group has previously reported, because treatment of hypoxic rats with the Rho kinase inhibitor attenuated both the pulmonary hypertension and the increases in total capillary length and volume.4 RhoA/Rho kinase signaling is certainly important in regulating endothelial cell morphogenesis, but how it might be involved in mediating the changes in pulmonary capillary structure in chronically hypoxic rats remains to be determined.

In summary, the report by Hyvel and colleagues provides further evidence that chronic hypoxia-induced pulmonary hypertension in rats is associated with an increase in total cross-sectional area of the pulmonary microvascular bed rather than with a loss of small pulmonary arteries, and that sustained Rho kinase–mediated vasoconstriction, rather than inward structural remodeling of pulmonary arteries and arterioles, is the primary determinant of the increased pulmonary vascular resistance and pulmonary hypertension. Adventitial and medial thickening of muscular pulmonary arteries and muscularization of the normally nonmuscular pulmonary arterioles occurs, but how this contributes to the increased resistance is unclear. It is apparent that much of the controversy regarding the extent of structural inward remodeling and rarefaction of peripheral pulmonary arteries in chronically hypoxic (and also monocrotaline injected) rats is attributable to the failure to eliminate vasoconstriction (vaso-

Schematic representation of the apparent exaggeration of medial thickening and inward structural remodeling caused by sustained vasoconstriction of hypertensive small pulmonary arteries in the chronically hypoxic rat whose vessels were not completely vasodilated before fixation (top). Recent studies indicate that a substantial part of the increased pulmonary vascular resistance in chronically hypoxic rats is attributable to Rho kinase–mediated vasoconstriction, and that the distal pulmonary arteries of hypertensive lungs pharmacologically vasodilated before fixation show only modest medial wall thickening and little reduction of lumen area (bottom). Thus, the near complete reversal of chronic hypoxic pulmonary hypertension with Rho kinase inhibitors is attributable to the fact that there is little functionally significant "inward" remodeling.

spasm) before and during lung fixation (Figure).4,5 In studies of resistance artery remodeling in systemic hypertension, it has long been appreciated that evaluation of the direct contribution of vascular structure to luminal narrowing, and therefore to vascular resistance, should be made only under conditions of complete lack of vascular tone, ie, under maximal vasodilation.40 The work of van Suylen et al and the McLoughlin group emphasizes that similar consideration should be applied to studies of vascular remodeling in pulmonary hypertension.3–5 Still, caution must be exercised in relating the findings obtained from relatively short-term hypoxic exposure of rats and mice to chronic human disease conditions where numerous other factors may play a role in the pulmonary hypertension and vascular remodeling associated with alveolar hypoxia.

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References


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