PULMONARY ARTERIAL HYPERTENSION (PAH) remains a clinically vexing problem considering the high morbidity and mortality that plagues this entity despite indication of improved survival after the introduction of more targeted therapy. While this syndrome is well described from a pathologic standpoint the mechanisms involved in the pulmonary vascular remodeling are less clear but are thought to involve endothelial dysfunction and proliferation of various cell types that comprise the wall of distal pulmonary vessels (3). The specific mechanisms leading to distal vessel obliteration and increased pulmonary vascular resistance have been particularly elusive for several decades (14), and although vasoconstriction as a primary event was the focus of research and targeted therapy in early studies, the PAH research community has witnessed a paradigm shift in recent years with a strong tendency to now view the PAH lesion as a quasi-cancerous process (12).

In this issue of the Journal of Applied Physiology, Schwenke et al. (8) bring a new tool for assessment of the distal pulmonary vasculature and shake an old specter, vasoconstriction. The investigators employ synchrotron radiation microangiography (SRA), a fairly novel and powerful technique, to more directly evaluate vascular tone in a classic animal model of PAH, monocrotaline (MCT)-challenged rats, providing new insight into regional specific responses of the pulmonary circulation in PAH. SRA allows for both the measurement and assessment of control of blood flow in vivo and collection of data on small arteries and arterioles in situ over time (10). Thus it can supply superior information on kinetic events relative to \textit{ex vivo} techniques. Since SRA has no focal plane, it can visualize both surface and penetrating vessels simultaneously unlike intravital microscopy, which is limited to assessment of vessel caliber at a single vascular plan. In dynamic organs such as the lung and heart, which are subject to motion, temporal subtraction allows for visualization of vessels as small as 80 μm in the lung (7, 9), and vessels as small as 20–30 μm have been studied in the entire mouse circulation (5). Thus SRA has greater temporal resolution than current magnetic resonance imaging techniques (i.e., vessels < 100 um, which are most affected by remodeling with new muscle formation and intimal proliferation in human PAH and animal models of the disease). In addition, and quite importantly, since there is no measure of PVR within these studies, it is impossible to determine to which extent the Rho-kinase inhibitor, fasudil, actually decreases PVR. The lack of measurement of cardiac output in this study is indeed a relative weakness. Thus, without demonstration of normalization of PVR, the relative contribution of fasudil-mediated vascular tone and vascular remodeling remains undefined.

Taking advantage of this approach, Schwenke et al. (8) are able to characterize both baseline differences in vascular architecture and dynamic changes in response to pharmacological interventions. More specifically, the authors investigated the effects of Rho-kinase inhibition on regional perfusion and vessel caliber (as determined essentially by measurement of internal diameter with microangiography). The balance between smooth muscle vasoconstrictive forces and vasodilatory forces are determinants of vascular tone. At the molecular level, these are in part regulated by the antagonistic functions of smooth muscle myosin light-chain kinase, which promotes constriction, and myosin light-chain phosphatase, which promotes dilation. Rho-kinase is a negative regulator of the vasodilatory phosphatase, and thus its activity promotes vasoconstriction (11). Schwenke et al. demonstrate that, in the context of MCT-induced PH, Rho-kinase inhibition restores blood flow to new regions of the lung. In addition to analysis of Rho-kinase effects on vascular recruitment and diameter, the authors provide, not surprisingly for this animal model, evidence of endothelial dysfunction (as assessed by a blunted pulmonary response to the endothelium-dependent vasodilator acetylcholine) in the setting of MCT-induced PAH. This study extends previous reports supporting a preponderant role for Rho-kinase-mediated vasoconstriction in MCT-induced pulmonary hypertension. Intriguingly the authors also unravel regional heterogeneity in endogenous nitric oxide synthase (NOS)-dependent tone, particularly in the setting of PAH. Furthermore, the authors provide sound evidence that Rho-kinase inhibition alters vascular recruitment and vessel caliber in vivo. The investigators should be commended for introducing a useful and powerful technique for assessing the pulmonary circulation more directly; their study, however, is not without limitations. The claim by the investigators that their collective observations provide evidence against the paradigm of pulmonary vessel “rarefaction” as a primary cause for the increase in vascular resistance in PAH is unfounded, or, to a minimum, it represents an overinterpretation of their data. While there is clear evidence that with Rho-kinase inhibition some vessels appear reperfused, this finding does not eliminate the contribution (to increased pulmonary vascular resistance, PVR) of rarefied vessels below the level of detection of the microangiography technique (i.e., vessels < 100 um, which are most affected by remodeling with new muscle formation and intimal proliferation in human PAH and animal models of the disease). In addition, and quite importantly, since there is no measurement of PVR within these studies, it is impossible to determine to which extent the Rho-kinase inhibitor, fasudil, actually decreases PVR. The lack of measurement of cardiac output in this study is indeed a relative weakness. Thus, without demonstration of normalization of PVR, the relative contribution of fasudil-mediated vascular tone and vascular remodeling remains undefined.

In Plato’s “Allegory of the Cave” from \textit{The Republic}, individuals chained to the wall of a cave watch and interpret the meaning of shadows (their reality) projected on the cave wall and generated by people and objects passing in front of a fire. Our pulmonary vascular research community has had its own shadows and distorted sense of reality, whether in experimental or human PAH. A lack of direct assessment of the pulmonary vasculature has hindered progress in interpreting
the actual degree of vascular remodeling (only assessed indirectly postmortem for humans and laboratory animals) from pathological cuts. While these findings clearly give a sense of the extent and severity of medial hypertrophy and intimal proliferation with the degree of muscularization and involvement of distal vessels, they are clearly limited by a two-dimensional and static insight into the pulmonary circulation. Both from an experimental and human standpoint, investigators have also been relying on indirect assessment of vascular changes, whether hemodynamics data obtained by cardiac catheterization or imaging of the right heart by echocardiography (and cardiac magnetic resonance imaging in human disease). While the heart, the first victim of the pulmonary vascular load and leading determinant of death when it fails under relentless load, should without doubt be the focus of further research aimed at understanding its function and as a potential important target for therapy, such efforts should not distract from developing methods to directly assess core changes in this disease, i.e., remodeling of the pulmonary vasculature. Thus the lack of a direct assessment of vascular changes (e.g., vessel size and diameter) and response has previously significantly hampered, in animal models of PAH, interpretation of changes, which was essentially based on extrapolating arguments from altered hemodynamics and pathological findings (the shadows in the allegory of the cave).

More direct methods have been notoriously lacking and have hindered progress in understanding the pathophysiology of the pulmonary circulation and potential changes operated by novel therapies. This is where the work by Schwenke and colleagues (8) is important and novel. It brings us one step out of the cave and closer to the fire where the action takes place, at least where animal models of PAH are concerned.

While this group has previously demonstrated the usefulness of the SRA technique to assess vessel internal diameters in both the chronic hypoxia and MCT rat models of PAH, the novelty of the present study lies essentially in the assessment of the specific role and contribution of Rho-kinase-mediated vasconstriction and endothelial dysfunction in the MCT model. This is an interesting and important study since, in addition to providing a useful tool to assess vessel morphometry in an intact animal model, it applies this technique to assess specific signaling pathways. However, whether specific vasodilation and Rho-kinase inhibition has a role beyond animal models of PAH remains unclear at this time. Vasoconstriction in the development of human PAH or as a contributor to increased PVR is controversial but thought to be present universally to some degree (6). Regarding specific Rho-kinase inhibition, inhaled fasudil was shown to have significant (but very modest) acute vasodilatory effects, as assessed by hemodynamic measurements, when given by inhalation to a small group of patients with idiopathic PAH and PAH associated with connective tissue diseases (1). The same authors had previously demonstrated a similar modest effect when given intravenously (2). An acute vasodilator effect was also demonstrated in patients with PAH associated with congenital heart disease (4). Therefore, while there is a plethora of information regarding the efficacy of fasudil in preventing and reversing (13) PAH in animal models, with now some useful insights into its specific effects on distal pulmonary vessels as elegantly provided by Schwenke et al. (8) in this issue, the effects of fasudil chronic therapy in humans remain to be determined.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES