Point:Counterpoint: Release of an endothelium-derived vasoconstrictor and RhoA/Rho kinase-mediated calcium sensitization of smooth muscle cell contraction are not the main effectors for full and sustained hypoxic pulmonary vasoconstriction

POINT: RELEASE OF AN ENDOTHELium-Derived VASOCONSTRICTOR AND RhoA/Rho KINASE-MEDIATED CALCIUM SENSITIZATION OF SMOOTH MUSCLE CELL CONTRACTION ARE THE MAIN EFFECTORS FOR FULL AND SUSTAINED HYPOXIC PULMONARY VASOCONSTRICTION

The ability of an organism, or an organ therein, to respond appropriately to episodes of hypoxia is a key evolutionary determinant. In mammalian lungs, the response to acute hypoxia involves constriction of the intrapulmonary arteries (IPA) in the hypoxic region of the lung. This response, termed hypoxic pulmonary vasoconstriction (HPV), effectively matches regional perfusion of the lung to regional ventilation and constitutes a pivotal homeostatic mechanism (25). HPV has, therefore, been the subject of intensive research for many years, although little consensus exists regarding its underlying sensor(s) and effector(s) mechanisms. Indeed, heated debates abound once one mentions trigger words and phrases such as “redox,” “reactive oxygen species,” “potassium channels,” etc., in relation to HPV (1, 28). This article focuses attention on another incendiary trigger phrase, namely “the endothelium.”

To consider the role of the endothelium in HPV, we must first define the hypoxic constrictor response itself. In vivo and in isolated blood-perfused lungs, HPV is usually immediate in onset and sustained. In contrast, HPV in isolated arteries is often biphasic, consisting of an initial transient constrictor response superimposed on a more slowly developing, sustained contraction (10). It has been argued that the latter, sustained, response is more physiologically relevant as it would appear important that HPV be a sustained response so that the redirection of blood flow in response to regional hypoxia is maintained for the duration of the hypoxic episode. It is important to note that although sustained HPV in isolated arteries is often referred to as “phase 2,” this does not mean that it is secondary to the transient phase of HPV. When pharmacological agents are used to block selectively the transient (i.e., “phase 1”) response (5, 20), it becomes apparent that sustained HPV in isolated arteries is immediate in onset, akin to that observed in perfused lungs and in vivo. In the review of Aaronson et al. (2), the authors tabulated the results of studies where the endothelium dependency of sustained HPV was examined. The final score of studies finding an endothelium dependency, be it partial or whole, versus those that found no role for the endothelium was 15 to 4. Given this convincing scoreline and the fact that it was spread over six species, I am sure that a betting person would place their wager on the proendothelium side of the debate!

Since the endothelial dependency of sustained HPV does not appear to be attributable to a reduction in the release of relaxing factors (2, 22), the case for an endothelium-derived constrictor factor(s) (EDCF) is compelling. Indeed, several studies have reported that an EDCF is released during HPV, although its precise identity remains elusive (5, 7, 21, 24). One possible candidate for this EDCF would be endothelin-1 (ET-1), and it has been postulated that ET-1 may play a priming role in some species (12, 22). However, ET-1 receptor antagonists are more often than not found to be ineffective in blocking HPV (2, 22) and ET-1 would appear to be a more likely an “agent provocateur” in chronic pulmonary hypertension (4).

Although the identity of the EDCF responsible for sustained HPV is unclear, more clarity exists concerning the mechanism by which it elicits contraction of the pulmonary vascular smooth muscle. It is apparent that the two principal determinants of smooth muscle tone, namely cytosolic Ca2+ ([Ca2+]i) and the sensitivity of the contractile apparatus to Ca2+, are elevated during sustained HPV (18). Specifically, following the transient rises in tension and [Ca2+]i associated with phase I of HPV, [Ca2+]i remains at a constant level (above that prior to hypoxia) while tension continues to rise (18, 20). The lack of correlation between tension and [Ca2+]i during sustained HPV is a classic example of Ca2+ sensitization. Endothelial denudation was found to abolish sustained HPV independent of any effect on the level of smooth muscle [Ca2+]i (17). These observations are wholly consistent with the elevation in smooth muscle [Ca2+], being an inherent property of pulmonary vascular smooth muscle, whereas the Ca2+ sensitization observed during sustained HPV is the purview of the EDCF.

At the time of this initial observation, one potential mediator of the Ca2+ sensitization observed during HPV, protein kinase C, was eliminated and it was not until the availability of the specific inhibitor of Rho-associated coiled coil-forming serine/threonine kinase (Rho kinase), Y-27632, that the activation of Rho kinase was first promulgated as a pivotal step in sustained HPV (19). Since the first isolation of Rho kinase (11), it has become apparent that, upon activation by the binding of the small monomeric G-protein RhoA, Rho kinase inhibits myosin light chain phosphatase (MLCP), resulting in Ca2+-independent contraction of vascular smooth muscle. Since this initial report, converging lines of evidence have pinpointed Rho kinase activation and subsequent inhibition of MLCP (27) as being a vital step in the initiation and maintenance of increased pulmonary artery pressure in response to acute hypoxia (6, 23), chronic hypoxia (6, 8, 13, 15), and pulmonary hypertensive states per se (3, 13, 14). Moreover, Rho-kinase inhibitors appear to show much promise as potential therapeutic agents in pulmonary hypertension (9). It is, therefore, possible that the activation of Rho kinase during sustained HPV represents the bridge between physiological HPV (i.e., that which occurs in response to brief and reversible hypoxia) and the pathophysiological consequences of chronic hypoxia.

It would seem logical to assume that since sustained HPV and the associated Ca2+ sensitization are both endothelium dependent then the activation of Rho kinase during hypoxia should be similarly endothelium dependent. However, it was reported that hypoxia can directly activate Rho kinase in...
cultured pulmonary artery smooth muscle cells (PASMC; Ref. 26). At first glance, this appears to scupper any idea of an EDCF-mediated increase in Rho kinase activation during sustained HPV. However, the increase in Rho kinase activity in cultured PASMC was a rather meager ~20% after 40 min of hypoxia, rising to a rather disappointing ~40% after 1 h. This is in stark contrast to the rather impressive ~260% increase in Rho kinase activity recently reported in isolated pulmonary arteries of the rat during sustained HPV (16). Moreover, this activation of Rho kinase in rat pulmonary arteries was endothelium dependent and in endothelium-denuded arteries the increase in Rho kinase activity was similar (~40%) to that reported for cultured PASMC. Furthermore, the translocation of RhoA to the plasma membrane of the pulmonary smooth muscle, a causal event in the activation of Rho kinase, was similarly dependent on the presence on an intact endothelium.

In closing, the case for the EDCF-RhoA/Rho kinase-Ca2+ sensitization axis being responsible for the maintenance of sustained HPV is solid. My most worthy opponent will probably try to convince the reader that the endothelium merely “modulates” the hypoxic constrictor response. However, when disruption of said axis results in removal of the hypoxic constrictor response, then “modulate” must surely be changed to “mediate”!

REFERENCES


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The great tragedy of Science—the slaying of a beautiful hypothesis by an ugly fact

Thomas H. Huxley (1825–1895)