Title: Hypoxic pulmonary hypertension: the paradigm is changing

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Sustained hypoxia caused by migration of native sea-level dwellers to high altitude or chronic lung disease leads to the development of increased pulmonary vascular resistance and pulmonary hypertension, a response to hypoxia that is unique to the pulmonary circulation. In susceptible individuals at high altitude (sub-acute and chronic mountain sickness) and in lung disease the resultant pulmonary hypertension causes significant disability and reduced life expectancy. For these reasons, investigation of the underlying mechanisms is an area of intense research activity. Classically the elevation in pulmonary vascular resistance in chronic hypoxic pulmonary hypertension has been attributed to structural remodelling of the vessels leading to thickening of the walls of the pulmonary arterioles which encroach into and narrow the vascular lumen; any vasoconstrictor component has been considered minor. However, more recent morphological evidence from experiments in chronically hypoxic rats suggested that lumen narrowing could not account for the increase in pulmonary vascular resistance or, at the very least, that the effect of this lumen narrowing may have been overestimated.

The identification of the RhoA/Rho-kinase (ROCK) signalling cascade as an important regulator of smooth muscle contraction and the development of the potent and selective ROCK inhibitors, including Y27632, provided the opportunity to gain new insights into processes in which altered smooth muscle contractility plays a key role, both physiologically and in disease processes (Uehata et al., 1997). Activation of the RhoA/ROCK pathway inhibits myosin light chain phosphatase activity thus enhancing the level of myosin light chain phosphorylation, promoting actin-myosin interaction and causing increased contraction. Effectively this increases the sensitivity of the contractile apparatus to Ca^{2+} and augments tension development at a given level of cytosolic Ca^{2+} (Uehata et al., 1997).

Prompted by these new discoveries, we began, in the early 2000s to explore the hypothesis that vasoconstrictor mechanisms, selectively altered in the lung, were significant contributors to the increase in pulmonary vascular resistance in pulmonary hypertension. We found that in the normal rat pulmonary circulation the RhoA-ROCK pathway is a greater contributor to vasoconstriction than it is in systemic vessels, demonstrating an important phenotypic difference in the regulation of vascular tone in the two circulations (Hyvelin et al., 2004). Almost simultaneously Fagan et al. reported the result of experiments in which Y27632 was used to examine the contribution of the RhoA-ROCK pathway to the elevation in pulmonary vascular resistance in the hypoxic mouse lung (Fagan et al., 2004). They found that ROCK activation contributes importantly to acute hypoxic pulmonary vasoconstriction (Fagan et al., 2004). Moreover, sustained ROCK inhibition *in vivo* attenuated the development of chronic hypoxic pulmonary hypertension (Fagan et al., 2004).

Taken together, these findings suggested for the first time that sustained vasoconstriction might play a more significant role in chronic hypoxic pulmonary hypertension than previously thought and that the RhoA-ROCK pathway might be important in this. Therefore we undertook a series of experiments to investigate the exact contributions of vasoconstriction and remodelling to the chronic hypoxia induced elevation in pulmonary vascular resistance in mice (Cahill et al., 2012). Using an isolated ventilated lung preparation we found that following long term hypoxic exposure, which established “fixed” pulmonary hypertension (i.e. not reversed by acute restoration of normal alveolar oxygen), acute ROCK inhibition using Y27632 reduced by half the increased pulmonary vascular resistance. Combining haemodynamic techniques with quantitative stereological analysis of the pulmonary vascular remodelling enabled us to also determine the degree to which structural remodelling of the pulmonary vascular wall and encroachment of the vessel wall into the lumen contributed to the increased resistance (Cahill et al., 2012). We found that the increase was due to equal contributions of sustained ROCK-dependent vasoconstriction and structural narrowing of the vascular lumen. This suggested that the role of sustained vasoconstriction in hypoxic pulmonary hypertension is considerably more important than previously thought.

Building on this identification of the importance of vasoconstriction in chronic hypoxic pulmonary hypertension, Wan et al. explored the effect of hypoxia on the regulation

of cytosolic calcium in pulmonary vascular smooth muscle (Wan et al., 2013). Increased cyctosolic Ca^{2+} is an important trigger for both pulmonary vasoconstriction and pulmonary artery smooth muscle cell proliferation and Ca^{2+} influx through voltage dependent calcium channels (VDCC) plays an important role in the regulation of cytoplasmic Ca^{2+} activity. Wan et al. demonstrated that sustained hypoxia induced functional upregulation of $Ca_v1.2$ (an L-type VDCC) and $Ca_v3.2$ (a T-type VDCC) in the pulmonary circulation without changing the expression of these channels in systemic vessels (Wan et al., 2013). Furthermore, they found that chronic hypoxia increased depolarization and agonist induced pulmonary vasoconstriction when compared to normoxic pulmonary vessels and that this increase was associated with higher cytosolic Ca^{2+} than in normoxic vessels. Pharmacological blockade of these two channels reduced the contractile responses to a greater extent in chronically hypoxic vessels than in control normoxic vessels (Wan et al., 2013). Their findings provide further experimental support for the hypothesis that chronic vasoconstriction is a major contributory mechanism in hypoxic pulmonary hypertension. Taken together these recent reports suggest that the mechanism of this chronic vasoconstriction is a lung selective increase in vascular smooth muscle cytosolic Ca^{2+} combined with an increased calcium sensitivity of the contractile apparatus mediated by the RhoA-ROCK pathway. Interestingly both pathways also mediate smooth muscle proliferation and may therefore contribute to the remodelling component of increased pulmonary vascular resistance.

Thus, the classic “text book” paradigm of chronic hypoxic pulmonary hypertension may need revision. Sustained vasoconstriction is an important contributor to the underlying increased pulmonary vascular resistance, although structural remodelling of the vessel also contributes. Not only do these findings have important implications for our basic understanding of this phenomenon, they also identify potential novel approaches to the treatment of pulmonary hypertension in disease. New pharmacological agents targeting the RhoA-ROCK pathway and calcium entry through the L- and T-type calcium channels whose expression and function are selectively enhanced in the hypertensive pulmonary circulation may be useful in the treatment of hypoxic pulmonary hypertension. Blockade could lead to an initial rapid vasodilation and reduction in resistance, followed by a later, more slowly developing, further reduction due to inhibition of remodelling. A potential advantage of inhibition

of such selectively upregulated pathways, either alone or in combination, is that this may permit effective therapy while minimising systemic vasodilatation and hypotension, a major limiting side effect of current therapies. Administration of antagonists by the inhaled route might further increase pulmonary selectivity. Additional studies are needed to establish the contribution of vasoconstriction to hypoxic pulmonary hypertension in humans and to identify the mechanisms that selectively regulate the underlying mechanisms in the hypoxic lung.

References

CAHILL, E., ROWAN, S. C., SANDS, M., BANAHAN, M., RYAN, D., HOWELL, K. & MCLOUGHLIN, P. (2012) The pathophysiological basis of chronic hypoxic pulmonary hypertension in the mouse: vasoconstrictor and structural mechanisms contribute equally. *Exp Physiol,* 97**,** 796-806.

FAGAN, K. A., OKA, M., BAUER, N. R., GEBB, S. A., IVY, D. D., MORRIS, K. G. & MCMURTRY, I. F. (2004) Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase. *Am J Physiol Lung Cell Mol Physiol,* 287**,** L656-64.

HYVELIN, J. M., O'CONNOR, C. & MCLOUGHLIN, P. (2004) Effect of changes in pH on wall tension in isolated rat pulmonary artery: role of the RhoA/Rho-kinase pathway. *Am J Physiol Lung Cell Mol Physiol,* 287**,** L673-84.

UEHATA, M., ISHIZAKI, T., SATOH, H., ONO, T., KAWAHARA, T., MORISHITA, T., TAMAKAWA, H., YAMAGAMI, K., INUI, J., MAEKAWA, M. & NARUMIYA, S. (1997) Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature,* 389**,** 990-4.

WAN, J., YAMAMURA, A., ZIMNICKA, A. M., VOIRIOT, G., SMITH, K. A., TANG, H., AYON, R. J., CHOUDHURY, M. S., KO, E. A., WANG, J., WANG, C., MAKINO, A. & YUAN, J. X. (2013) Chronic hypoxia selectively enhances L- and T-type voltage-dependent Ca2+ channel activity in pulmonary artery by upregulating Cav1.2 and Cav3.2. *Am J Physiol Lung Cell Mol Physiol,* 305**,** L154-64.

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