Cellular responses to hypoxia in the pulmonary circulation

Seema O Brij, Andrew J Peacock

Oxygen is essential for aerobic respiration to produce ATP. It is possible that all cells are able to sense changes in oxygen content or tension but the mechanisms by which different cell types sense these changes remain poorly understood. The glomus cells of the carotid body, neuroepithelial bodies of the airway mucosa, and the cells of the pulmonary vasculature in animals and humans are fast responding systems and exhibit specific physiological responses when exposed to low oxygen tensions. In addition, certain gene systems—erythropoietin (EPO) is the classic example—also respond to hypoxia. However, the highest levels of EPO are seen in patients with aplastic anaemia, suggesting that EPO upregulation is proportional to oxygen concentration and not oxygen tension.

Alveolar hypoxia causes pulmonary vasoconstriction and, if this hypoxic period is prolonged, there is vascular remodelling which renders the vessel indistensible such that fixed pulmonary hypertension ensues. The processes by which hypoxic pulmonary vasoconstriction and hypoxic vascular remodelling occur need to be fully investigated if the pathological end points are to be reversed in patients exposed to hypoxia, whether secondary to lung disease or to residence at high altitude.

In non-anaesthetised animals the characteristic cardiac and respiratory responses to acute hypoxia are well documented. The respiratory rate, heart rate, and cardiac output all increase to maintain arterial oxygen tension and ensure adequate tissue oxygenation. These responses are thought to be mediated by the peripheral arterial chemoreceptors. In the newborn animal these circulatory and respiratory responses to hypoxia are less developed but by six weeks the adult response is intact. In the mammalian fetus the high pulmonary vascular tone ensures a right to left shunt of blood through the patent foramen ovale and ductus arteriosus, thereby bypassing the lungs. This active vasomotor response persists in adulthood but only to maximise perfusion-ventilation matching in the face of regional hypoxia.

Hypoxia decreases the systemic vascular resistance and yet the pulmonary vascular resistance increases. In 1946 Von Euler and Liljestrand demonstrated in the anaesthetised cat that pulmonary artery pressure was inversely proportional to oxygen tension. Hypoxic pulmonary vasoconstriction (HPV) is intrinsic to the lung and can be demonstrated in isolated perfused lungs, small pulmonary artery rings, and in isolated vascular smooth muscle cells. This response is complicated. Due to their elastic and recoil properties the largest proximal arteries act as conduit vessels. Conduit pulmonary arteries have a biphasic response to hypoxia with an initial constriction followed by a sustained relaxation. The smaller muscularised arteries act as resistance vessels and constrict in response to hypoxia.

This is the response known as hypoxic pulmonary vasoconstriction (HPV). HPV is specific to alveolar hypoxia and does not occur when there is hypoxaemia without alveolar hypoxia—for example, in a right to left intracardiac shunt.

Hypoxia, oxygen sensing, and vasoconstriction

Oxygen sensing has been well studied but the mechanism by which oxygen is detected at a cellular level is still uncertain. The smooth muscle cell of the pulmonary vasculature, the glomus cell of the carotid body, and the neuroepithelial bodies of the airway all elicit a fast response to hypoxia. There is increased activity within seconds to minutes: the pulmonary smooth muscle cell constricts, the glomus cell releases dopamine, and there is enhanced secretion of dense core vesicles containing serotonin from neuroepithelial bodies. The common link in their cellular response to hypoxia is a reduction in whole cell potassium current and a rise in intracellular calcium.

HYPOXIA AND CELLULAR ION CHANNELS

The response of individual ion channels can be studied using patch clamp techniques. In essence, a micro-electrode is placed against the surface of a cell and suction is applied to the electrode creating a high resistance seal. The sealed patch electrode can then be used to monitor the activity of whatever channels happen to be trapped inside the seal. The patch of membrane can be studied in situ or removed from the cell so that the composition of the solution in contact with the membrane may be manipulated. In a similar way whole cell current can also be measured.

Membrane potential is normally maintained between −40 and −60 mV. This transmembrane potential is largely controlled by potassium (K) channels. When K channels are opened positively charged ions move from the interior to the exterior of the cell. This results in the interior of the cell becoming relatively more negative compared with the exterior. This is membrane hyperpolarisation. If the K channels are blocked the interior of the cell becomes less negative and membrane depolarisation results.

At least four classes of K channels have been identified in vascular smooth muscle cells: voltage dependent (Kv), which delay ed recti-
HYPOXIA AND THE CAROTID BODY

The carotid body is known to sense hypoxia and is responsible for the hyperventilation seen in non-anaesthetised mammals. These receptors consist of two types of glomus cells (I or II) and are neuroectodermal in origin. Type I (chief) cells contain cytosolic granules rich in dopamine surrounded by type II (sustentacular) cells which are glia-like and are responsible for the carotid body hyperplasia seen in patients with chronic hypoxic lung disease and at altitude. Carotid bodies are vascular structures for their size and have a small arterial-venous oxygen difference in spite of their high metabolic rate. They respond to changes in arterial oxygen tension but not to anaemia or reduced blood flow. If the carotid bodies are removed the response to hypoxia is depression of respiration via inhibition of the central respiratory centres.

Patch clamp techniques have demonstrated selective inhibition of a delayed rectifier potassium channel in rabbit glomus type I cells when exposed to hypoxia. The induced membrane depolarisation opens a voltage dependent (L-type) calcium channel which increases cytosolic calcium and facilitates neurotransmitter release. Type I glomus cells synthesise and secrete dopamine during hypoxia. Activated sensory terminals of afferent sensory fibres of the sinus nerve (a branch of the glossopharyngeal nerve) relay information to respiratory centres in the brainstem and stimulate ventilation.

HYPOXIA AND THE NEUROEPITHELIAL BODIES

Pulmonary neuroepithelial bodies are found throughout the airway mucosa in human and animal lungs. They consist of islands of amine and peptide containing vesicles which are released upon hypoxic stimulation. These neuroepithelial bodies are therefore similar to other chemoreceptors such as the carotid body and the taste bud.

Patch clamp techniques have demonstrated an oxygen sensitive potassium channel in neuroepithelial body cells. During hypoxia potassium channels close, inducing membrane depolarisation. Voltage dependent calcium channels are opened which increases cytosolic calcium and facilitates neurotransmitter release. This suggests that neuroepithelial body cells may be transducers of the hypoxic stimulus but their role remains uncertain.

HYPOXIA AND PULMONARY SMOOTH MUSCLE CELLS

The mechanism by which the cells of the pulmonary vasculature sense hypoxia is uncertain but there is a likely role for the oxygen sensitive ion channels. In cultured pulmonary artery smooth muscle cells hypoxia alters potassium channel activity in a similar fashion to that seen in type I cell of the carotid body and neuroepithelial bodies. This reduction in whole cell potassium current may provoke membrane depolarisation and calcium influx. Calcium influx has been shown to initiate pulmonary smooth muscle cell contraction which is clinically manifest as hypoxic pulmonary vasoconstriction.

Systemic vascular smooth muscle cells from cerebral, mesenteric, and renal circulations do not inhibit potassium current in response to hypoxia. In systemic circulations exposed to hypoxia it is thought that KATP channels are opened, thereby hyperpolarising the membrane and inhibiting calcium influx. Thus there is smooth muscle cell relaxation which is clinically manifest as systemic vasodilatation.

In the pulmonary artery the distribution of K channel subtypes in small distal resistance arteries differs from those in the larger proximal conduit arteries. Resistance artery smooth muscle cells have a higher density of KCa rectifier potassium channels which exhibit oxygen sensitivity by their K channel inhibition. Conversely, in the conduit vessels there is a higher proportion of KATP channels which increase whole cell potassium current on hypoxic exposure. Conduit and resistance myocytes also differ in their voltage gated (L-type) calcium channel response to hypoxia. In the conduit arterioles hypoxia inhibits calcium current whereas in resistance arterioles hypoxia increases calcium influx.

The heterogeneity in distribution of K and calcium channels may explain some of the discrepancies in vascular reactivity noted between resistance and conduit arteries in response to hypoxia. Resistance arterioles respond to hypoxia by inhibition of whole cell potassium current and depolarisation of the membrane leading to calcium influx and constriction. Conduit arteries are more akin to systemic
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Pulmonary vascular cell proliferation and hypoxia

The rise in intracellular calcium is thought to activate the cellular mechanisms which result not only in pulmonary smooth muscle cell contraction but also in the proliferative response and remodelling which occurs in all three layers in the vessel wall in response to hypoxia.

THE HYPOXIC Proliferative RESPONSE

Chronic hypoxia is characterised by a sustained increase in pulmonary vascular tone which in part is maintained by vascular remodelling. The duration of the hypoxia, its severity, and the time of life at which the exposure is experienced all serve to modify the outcome. In addition, each cell type in the pulmonary arterial wall—the endothelial cell, the smooth muscle cell and the adventitial fibroblast—has a specific growth response to hypoxia.

Meyrick and Reid found that within 24 hours of hypoxia in vivo there was an increase in proliferation of the endothelial cell, medial smooth muscle cell, and adventitial fibroblast. However, the hypoxic proliferative response was different in proximal hilar vessels and distal intra-alveolar vessels. In the hilar vessels hypoxia caused a threefold increase in endothelial cell proliferation. There was minimal change in the medial smooth muscle in comparison with the adventitial fibroblasts which showed an eightfold increase in proliferation. In small intra-alveolar arteries hypoxia stimulated smooth muscle cell proliferation and the muscularisation of previously non-muscular small arteries. The endothelial cell and adventitial fibroblasts also showed increased proliferation, though not as striking as in the proximal vessels.

The adventitial fibroblast is unique in that it proliferates directly in response to hypoxia. This response is augmented with the addition of growth factors. Marked increases in fibroblastic matrix deposition—namely, collagen and elastin—has been observed under conditions of chronic hypoxia which contribute to the indistinguishability of the vessel wall.

HYPOXIC PROLIFERATION AND INTRACELLULAR SIGNALLING

Protein kinase C (PKC) is a family of intracellular enzymes known to play a significant role in the signal transduction of normal cellular proliferation. PKC is a calcium-dependent enzyme and, once activated, it stimulates DNA synthesis in systemic artery smooth muscle cells and influences Na⁺-H⁺ exchange.

PKC is known to be important in the growth of pulmonary vascular cells. Pulmonary myocytes will not proliferate in response to hypoxia unless PKC is activated. Cultured fetal and neonatal pulmonary adventitial fibroblasts exhibit increased expression of PKC during hypoxic growth (fig 1).

PKC is also activated by the lipid second messenger diacylglycerol which is generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP₂). The other by-product of this reaction, inositol-1,4,5-triphosphate (IP₃), translocates to a receptor on the endoplasmic reticulum triggering the release of intracellular calcium. Hypoxia enhances the proliferation and generation of IP₃ in pulmonary artery fibroblasts but not in those from the mesenteric circulation (fig 1).

The mitogen activated protein kinase (MAP kinase) pathway is also important in the signal transduction of normal proliferation and exerts its effects downstream of PKC. MAP kinases are activated by multiple phosphorylation events. A major advance in the understanding of intracellular signalling events in hypoxia is that heat shock, osmotic shock, antioxidants, ultraviolet light and DNA damaging agents are able to activate MAP kinases. Work in our laboratory has shown that hypoxia also stimulates the “stress activated” MAP kinases which may therefore be involved in the hypoxic proliferative response.

HYPOXIA AND THE UPREGULATION OF GROWTH MITOGENS

Hypoxia increases the proliferation of all three cell types in the pulmonary arterial wall. Many of the growth factors which are known to cause proliferation in pulmonary vascular cells are also vasoconstrictors—for example, endothelin...
is far from complete. How these cellular processes result in the physiological end points observed, the respiratory and cardiovascular responses, the regulatory vasomotor responses, the pulmonary vascular remodelling is still being investigated. Clearly, a better understanding of the effects of hypoxia is needed if the development of pulmonary hypertension in subjects with hypoxic lung disease or those living at altitude is to be prevented.


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