

## REVIEW ARTICLE

## MECHANISMS OF DISEASE

## Acute Oxygen-Sensing Mechanisms

E. Kenneth Weir, M.D., José López-Barneo, M.D., Ph.D., Keith J. Buckler, Ph.D.,  
and Stephen L. Archer, M.D.

From the Department of Medicine, Minneapolis Veterans Affairs Medical Center and University of Minnesota, Minneapolis (E.K.W.); the Laboratorio de Investigaciones Biomédicas, Hospital Virgen del Rocío, Universidad de Sevilla, Seville, Spain (J.L.-B.); the Department of Physiology, Oxford University, Oxford, England (K.J.B.); and the Department of Medicine and Physiology, University of Alberta, Edmonton, Alta., Canada (S.L.A.). Address reprint requests to Dr. Weir at the VA Medical Center 111C, 1 Veterans Dr., Minneapolis, MN 55417, or at weir002@umn.edu.

N Engl J Med 2005;353:2042-55.  
Copyright © 2005 Massachusetts Medical Society.

JOSEPH PRIESTLEY, ONE OF THE THREE SCIENTISTS CREDITED WITH THE discovery of oxygen, described the death of mice that were deprived of oxygen. However, he was also well aware of the toxicity of too much oxygen, stating, “For as a candle burns much faster in dephlogisticated [oxygen-enriched] than in common air, so we might live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve.”<sup>1</sup>

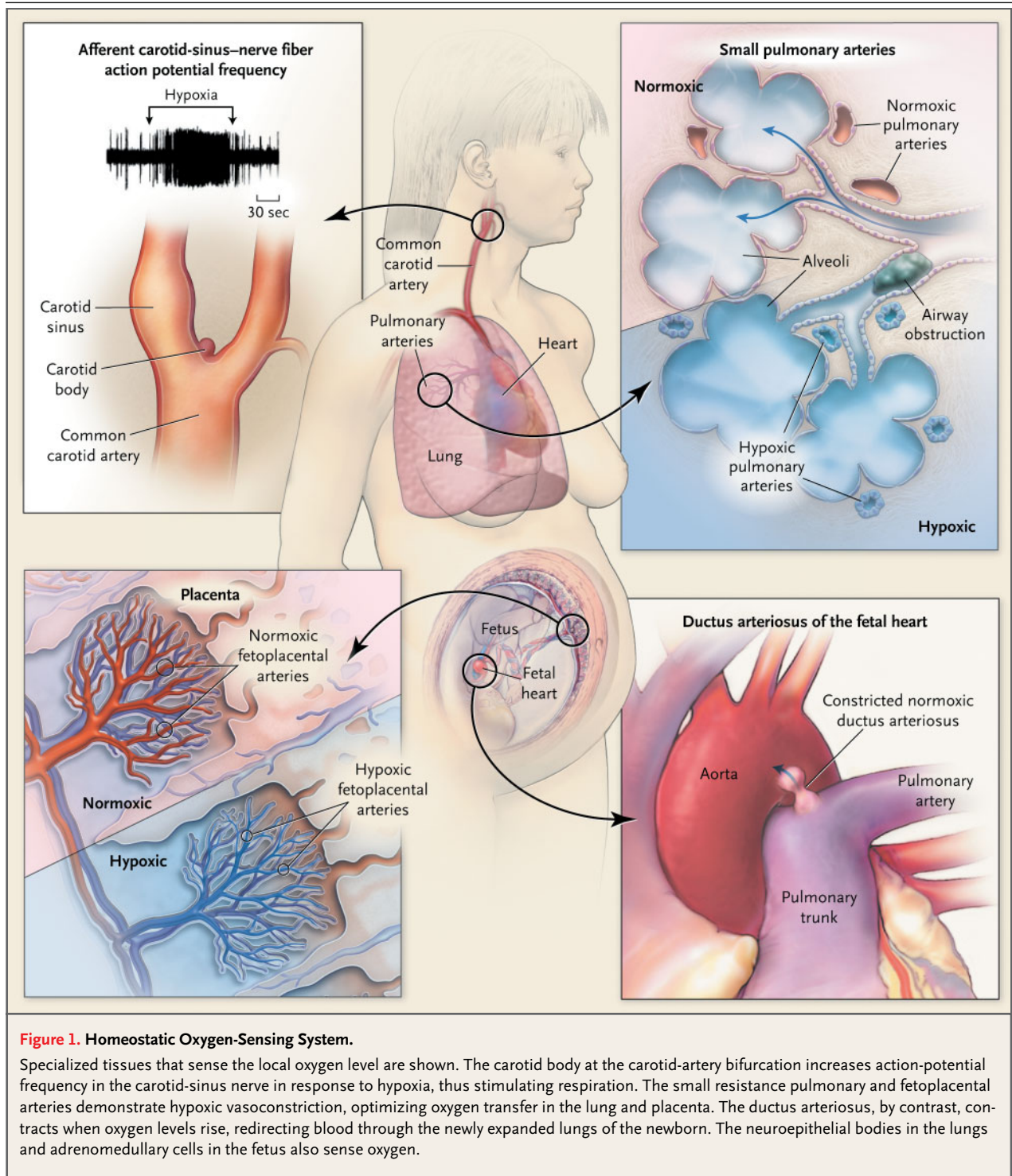
In this review we examine the remarkable mechanisms by which different organs detect and respond to acute changes in oxygen tension. Specialized tissues that sense the local oxygen tension include glomus cells of the carotid body, neuroepithelial bodies in the lungs, chromaffin cells of the fetal adrenal medulla, and smooth-muscle cells of the resistance pulmonary arteries, fetoplacental arteries, systemic arteries, and the ductus arteriosus. Together, they constitute a specialized homeostatic oxygen-sensing system. Although all tissues are sensitive to severe hypoxia, these specialized tissues respond rapidly to moderate changes in oxygen tension within the physiologic range (roughly 40 to 100 mm Hg in an adult and 20 to 40 mm Hg in a fetus) (Fig. 1).

## HYPOXIC PULMONARY VASOCONSTRICTION

In fetal life, the pulmonary vascular bed has a high resistance to blood flow. Consequently, oxygenated blood returning from the placenta is diverted from the unventilated lungs and across the foramen ovale and ductus arteriosus. At birth, when air breathing begins, the lungs expand and oxygen levels rise. With reversal of fetal hypoxic pulmonary vasoconstriction, the pulmonary vessels dilate and the ductus arteriosus constricts, thereby establishing the transition from the fetal to the neonatal circulation.

After birth, hypoxic pulmonary vasoconstriction remains important, because it reduces perfusion of poorly ventilated areas of lung, and in so doing it decreases the shunting of desaturated, mixed venous blood to the systemic circulation. Inhibition of hypoxic pulmonary vasoconstriction reduces the systemic arterial oxygen tension, particularly in small-airway disease.<sup>2</sup> Moreover, as was first demonstrated in humans in 1947,<sup>3</sup> the intensity of hypoxic pulmonary vasoconstriction depends on the severity and duration of alveolar hypoxia.<sup>4,5</sup>

The endothelium produces vasodilators, such as nitric oxide and prostacyclin, and vasoconstrictors, such as endothelin and thromboxane A<sub>2</sub>; these molecules from endothelial cells modulate hypoxic pulmonary vasoconstriction, but the ability of small pulmonary vessels to contract in response to hypoxia resides in their smooth-muscle cells.<sup>6</sup> Three sites in these cells are involved in the mechanism of hypoxic pulmonary vasoconstriction: the membrane, the sarcoplasmic reticulum, and the contractile apparatus.

**THE SMOOTH-MUSCLE-CELL MEMBRANE**

At the smooth-muscle-cell membrane in the pulmonary artery, hypoxic inhibition of the outward potassium current causes depolarization of the

membrane and entry of calcium through L-type voltage-gated calcium channels (see the glossary for definitions of terms).<sup>7,8</sup> The membrane potential, and therefore control of voltage-gated calcium

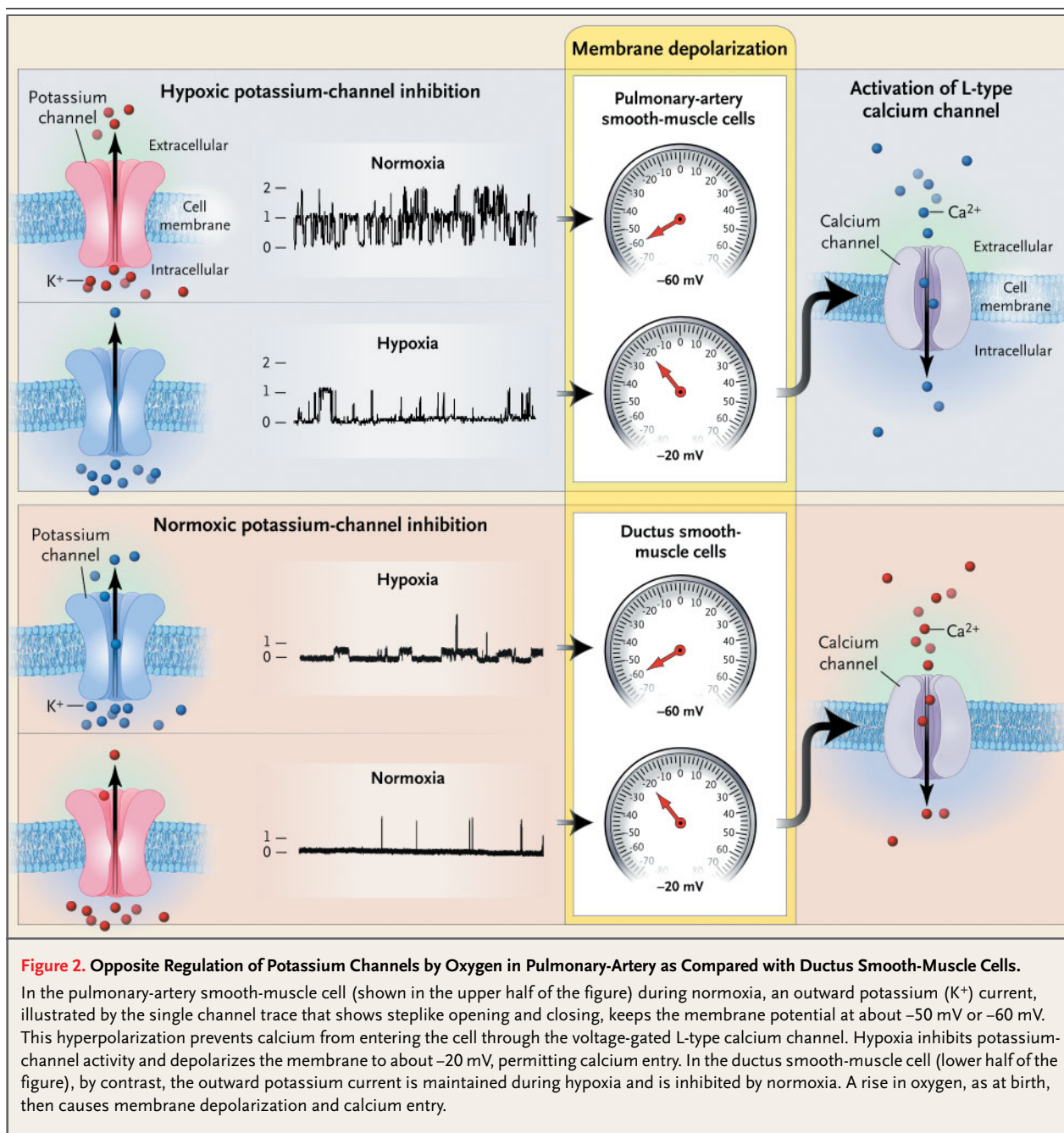
## Glossary

- Membrane depolarization:** Potassium-channel inhibition, whether by a chemical or by oxygen tension, leads to accumulation of positively charged potassium ions ( $K^+$ ) within the cell, and this positive shift in membrane potential (i.e., depolarization from  $-60$  mV) activates voltage-gated, L-type calcium channels, promoting calcium influx.
- Membrane potential:** The voltage difference across the cell plasma membrane that results from a charge separation of ions. There is a balance between negatively charged macromolecules trapped within the cell and the asymmetric distribution of ions. More potassium ions ( $K^+$ ) and anionic proteins are within the cell and more  $Cl^-$  and  $Na^+$  ions are outside. The ions can cross the membrane only through selective pores, called ion channels, or by means of pumps and transporters. Ion-channel opening, especially that of  $K^+$  channels, largely controls membrane potential. At a certain point (the equilibrium potential) the tendency of an ion to exit or enter the cell based on its concentration gradient is exactly balanced by the electrostatic (charge) forces.
- Mitochondrial electron transport chain:** A cascade of proteins and iron-sulfur complexes within the inner mitochondrial membrane that shuttle electrons derived from NADH oxidation down the redox potential gradient to molecular oxygen. In addition to driving ATP synthesis, the electron transport chain creates a leak of reactive oxygen species, owing to uncoupled electron transfer.
- Potassium channels:** Tetrameric proteins in the cell membrane. They have a highly conserved pore region with a potassium-recognition sequence that determines ion specificity. There is great diversity in the N and C terminals of potassium channels that regulate channel expression and gating. The major classes of potassium channels include voltage-gated, inward rectifier, and two-pore.  $\beta$  subunits associate with many potassium channels and alter their expression and kinetics.
- Reactive oxygen species:** Substances, created in small amounts in the course of oxygen metabolism, that include stable diffusible substances such as hydrogen peroxide and unstable toxic radicals (which have an unpaired electron), such as superoxide and hydroxyl radicals. Originally recognized as toxic products of radiation, the reactive oxygen species are now recognized as redox-generated messengers that change the function or conformation of target molecules, such as sulfhydryl-rich potassium channels and also control enzymes, such as deacetylases that control gene transcription and apoptosis. Reactive oxygen species are produced from the mitochondrial electron transport chain (complexes I and III) and oxidases, such as NADPH oxidase.
- Redox:** A contraction for “reduction–oxidation.” Redox reactions are most simply defined as the transfer of electrons between pairs of chemical species so that the electron donor is “oxidized” and the recipient is “reduced.”
- RhoA and Rho kinase:** Rho kinase is a serine–threonine kinase that is activated by the GTP-binding protein RhoA. Rho kinase phosphorylates the regulatory subunit of smooth-muscle myosin phosphatase and inhibits phosphatase activity, thereby mediating calcium sensitization of the contractile apparatus. Sensitization causes sustained contraction of vascular smooth muscle in response to hypoxia and vasoconstrictor agonists, even after calcium concentrations decline. Inhibition of Rho kinase relaxes most arteries and counteracts vasoconstriction.
- Sarcoplasmic reticulum:** A membranous intracellular organelle that participates in calcium homeostasis by buffering (accumulating) intracellular calcium. During relaxation calcium is sequestered, and during contraction it is released. Calcium handling is controlled by sarcoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA) and two types of  $Ca^{2+}$  channels, the ryanodine receptor and the inositol 1,4,5-triphosphate receptor.
- Store-operated channels:** Depletion of intracellular calcium stores results in capacitative calcium influx via store-operated channels. On a molecular level, store-operated channels appear to be encoded by transient receptor potential (TRP) genes. The store-operated channels are involved in regulation of vascular tone and cell proliferation.
- Two-pore acid-sensitive potassium channels (TASK channels):** A family of potassium channels that, unlike most others, has two pore domains. They are not gated by voltage but conduct a basal leak current at negative membrane potentials that contributes to resting membrane potential. TASK channels are inhibited by acidic pH and activated by certain anesthetic agents, such as halothane.
- Voltage-gated (L-type) calcium channels:** Large, long-lasting calcium channels that are voltage activated. When open, they permit influx of calcium into excitable cells down the calcium concentration gradient (2 mM extracellular vs. 100 nM intracellular). L-type channels are critical to the control of vascular tone but are also involved in neurotransmitter release, gene expression, and cell proliferation. They are blocked by dihydropyridines (e.g., nifedipine), phenylalkylamines, and benzothiazepines.
- Voltage-gated potassium (Kv) channels:** Kv channels have an arginine-and-lysine-rich voltage sensor in their fourth transmembrane domain region. This sensor changes in conformation with membrane depolarization, thus opening the channel. There are several families of Kv channels (Kv1 through Kv9), each with isoforms (e.g., Kv1.1 through Kv1.6).

channels in the membrane of the smooth-muscle cell, is largely determined by the movement of potassium across the membrane from a high concentration inside the cell (145 mM) to a low concentration outside the cell (5 mM). At the resting membrane potential (about  $-60$  mV) these calcium channels are mostly closed. Figure 2 shows the sequence of inhibition of potassium current, membrane depolarization, and entry of calcium ions elicited by hypoxia.<sup>7,8</sup> Hypoxia inhibits potassium current and depolarizes smooth-muscle cells in the

pulmonary arteries, but it does not have these effects in smooth-muscle cells from vascular beds that dilate in response to hypoxia (e.g., those of the kidney or mesentery). Inhibition of potassium current is proportional to the severity of hypoxia<sup>9</sup> and is more prominent in small resistance pulmonary arteries (diameter,  $<500$   $\mu$ m) than in large extraparenchymal pulmonary arteries.<sup>10</sup>

A variety of potassium channels in smooth-muscle cells of the pulmonary arteries are sensitive to acute changes in oxygen.<sup>11</sup> In the fetus, the cal-



cium-sensitive potassium channel ( $K_{Ca}$ ) is the predominant oxygen-sensitive channel.<sup>12</sup> After birth, a shift to several voltage-gated potassium channels ( $K_v$ ; this nomenclature refers to  $K$  channel, voltage-dependent) occurs.<sup>11</sup> For instance, hypoxia inhibits  $K_v1.5$ , which has been cloned from human pulmonary arteries,<sup>13</sup> and hypoxic pulmonary vasoconstriction is diminished in mice that lack this channel.<sup>11</sup> Acute hypoxic pulmonary vasoconstriction is

blunted in rats previously exposed to chronic hypoxia,<sup>14</sup> and chronic hypoxia decreases the oxygen-sensitive component of potassium current and the expression of  $K_v1.5$  and  $K_v2.1$  in smooth-muscle cells of the pulmonary arteries.<sup>15-18</sup> These findings, together with the observation that the diminution of hypoxic pulmonary vasoconstriction by chronic hypoxia in rats can be restored by aerosol transfection of human  $K_v1.5$ ,<sup>19</sup> have established a role for

Kv channels, and particularly Kv1.5, in the mechanism of hypoxic pulmonary vasoconstriction. Kv2.1 and Kv3.1b and another potassium channel, two-pore acid-sensitive potassium channel type 1 (TASK-1), may also be involved.<sup>13,20,21</sup> Vasoconstriction caused by hypoxic inhibition of potassium current must involve membrane depolarization, but even after marked depolarization has been induced in ring segments of pulmonary arteries by the addition of 80 mM of potassium chloride, hypoxia causes further contraction.<sup>22</sup> This observation indicates that hypoxic pulmonary vasoconstriction also involves other mechanisms.

#### RELEASE OF CALCIUM FROM THE SARCOPLASMIC RETICULUM

The L-type calcium-channel blocker nifedipine reduces the severity of acute hypoxic pulmonary vasoconstriction provoked in patients with chronic lung disease by more than 50 percent.<sup>23</sup> Whereas most of the calcium involved in hypoxic pulmonary vasoconstriction comes from outside the cell, some comes from internal stores.<sup>9</sup> The observation that depletion of calcium in the intracellular sarcoplasmic reticulum reduces hypoxic pulmonary vasoconstriction led to the conclusion that hypoxia normally causes release of calcium from this store.<sup>24-26</sup> Release then leads to repletion of the sarcoplasmic reticulum by influx of calcium into the smooth-muscle cell that occurs through store-operated calcium channels.<sup>27-30</sup> Inhibitors of these channels block calcium influx into the smooth-muscle cells of the pulmonary arteries that are stimulated by hypoxia<sup>27</sup> and prevent hypoxic pulmonary vasoconstriction.<sup>28</sup>

In summary, the response of the smooth-muscle cells in the pulmonary arteries to acute hypoxia begins within seconds and involves inhibition of potassium current, membrane depolarization, and calcium entry through L-type calcium channels; it also involves calcium release from the sarcoplasmic reticulum and calcium repletion through store-operated channels.

#### RhoA/Rho-KINASE AUGMENTATION OF HYPOXIC PULMONARY VASOCONSTRICTION

Contraction of vascular smooth-muscle cells is initiated by phosphorylation of myosin light chain, which is induced by the calcium-calmodulin-dependent myosin light-chain kinase. Dephosphorylation is mediated by myosin light-chain phosphatase. Consequently, vascular tone is modulated

by the balance of activities between the kinase and the phosphatase. At any given level of cytosolic calcium, the degree of contraction of smooth-muscle cells caused by the interaction of actin with myosin can be increased by inhibition of the myosin light-chain phosphatase.<sup>31</sup> In the contractile response to hypoxia, the dissociation between a sustained level of cytosolic calcium and gradually increasing contraction of small pulmonary arteries is important.<sup>32</sup> Hypoxia, acting through the small G protein RhoA, stimulates Rho kinase,<sup>33</sup> which inhibits myosin light-chain phosphatase, thereby increasing phosphorylation of the light chain and augmenting contraction. Consequently, Rho kinase increases hypoxic pulmonary vasoconstriction,<sup>34</sup> and Rho-kinase inhibitors reduce hypoxic pulmonary vasoconstriction.<sup>35,36</sup>

#### NORMOXIC CONSTRICTION OF THE DUCTUS ARTERIOSUS

The importance of oxygen in initiating closure of the ductus is underlined by the occurrence of six times as many cases of patent ductus arteriosus in babies born at high altitude (approximately 4000 m) as in those born at sea level.<sup>37</sup> Oxygen-induced membrane depolarization in the ductus arteriosus occurs because an increase in oxygen tension inhibits Kv channels (Fig. 2).<sup>38,39</sup> Membrane depolarization of smooth-muscle cells in the ductus, as in those in pulmonary arteries, causes the entry of calcium, which can be inhibited by L-type calcium-channel blockers.<sup>38,40</sup>

In a process analogous to the reduction of hypoxic pulmonary vasoconstriction in chronic hypoxia, rings of ductus arteriosus tissue selectively fail to constrict in response to oxygen after several days in culture under normoxic conditions.<sup>39</sup> The messenger RNA (mRNA) for Kv1.5 and Kv2.1 is diminished in this so-called chronic normoxia model, and experimental *ex vivo* gene therapy with Kv1.5 or Kv2.1 restores much of the contractile function of the ductus rings.

#### HYPOXIC STIMULATION OF THE CAROTID BODY

Hypoxemia in the systemic blood (arterial oxygen pressure, <60 mm Hg) leads to neurosecretion by the type 1, or glomus, cells of the carotid body. Activation of the carotid body causes the sensation of breathlessness experienced at high altitudes. Re-

lease of acetylcholine and ATP from the carotid body stimulates sensory-nerve endings in the carotid-sinus nerve and activates the respiratory center (Fig. 3). The first demonstration of hypoxic inhibition of potassium current was made in the glomus cell.<sup>41</sup> Subsequently, Kv, K<sub>Ca</sub>, and TASK-like glomus-cell potassium channels were shown to be oxygen-sensitive.<sup>42-45</sup> As in the pulmonary artery, the relevant oxygen-sensitive channel varies with species and age. Hypoxic inhibition of these potassium channels causes membrane depolarization and influx of calcium. The increase in cytosolic calcium induced by hypoxia does not occur in the absence of extracellular calcium or without membrane depolarization (Fig. 3).<sup>46,47</sup> L-type calcium-channel blockers prevent much of the increase in cytosolic calcium in glomus cells that is caused by a change in oxygen tension.<sup>46</sup> The increase in cytosolic calcium is closely related to the secretion of dopamine (a neurotransmitter abundant in glomus cells) (Fig. 3),<sup>48</sup> and hypoxia-induced neurosecretion is prevented by removal of extracellular calcium.<sup>49</sup>

The homeostatic sequence elicited by hypoxia (potassium-channel inhibition, membrane depolarization, calcium entry, and neurosecretion) is generally accepted. It is less clear which channel initiates the membrane depolarization, thereby bringing the membrane potential into the range in which hypoxic inhibition of other potassium channels contributes to the depolarization. TASK-like channels are non-voltage-gated channels that are active at the resting membrane potential (less than -60 mV) of the glomus cell and could fulfill this role. They are inhibited by hypoxia, which leads to membrane depolarization in acutely isolated glomus cells. The observation that tetraethylammonium and iberiotoxin, both inhibitors of K<sub>Ca</sub> channels, cause neurosecretion in cultured slices of the carotid body of rats also indicates that K<sub>Ca</sub> or Kv channels, or both, contribute to the setting of resting membrane potential. This effect of potassium-channel inhibition has been exploited clinically to enhance respiration. The respiratory stimulant doxapram mimics hypoxia by inhibiting both K<sub>Ca</sub> and Kv currents in glomus cells.<sup>50</sup>

The ion-channel modulation and loss of responsiveness to oxygen, elicited in the pulmonary arteries by chronic hypoxia and in the ductus arteriosus by chronic normoxia, are recapitulated in the carotid body. There the glomus cells of neonatal rats raised under hypoxic conditions show reduced potassium current and hypoxia-induced depolarization.<sup>43</sup> The

loss of oxygen-sensing ability caused by chronic hypoxia may explain the body's failure to increase ventilation in response to acute hypoxia in those living at high altitudes.<sup>51</sup> In the pulmonary artery, the decreased expression of potassium channels is in part signaled by hypoxia-inducible factor 1 $\alpha$  (usually referred to as HIF-1 $\alpha$ ),<sup>52</sup> through its actions as a regulator of gene transcription.

---

#### OTHER EXAMPLES OF OXYGEN SENSING BY POTASSIUM CHANNELS

---

##### THE PLACENTA

Fetal arteries in the placenta constrict in response to hypoxia, diverting fetal blood to cotyledons with better maternal perfusion.<sup>53,54</sup> This response optimizes the transfer of placental oxygen, which, if impaired, can cause intrauterine growth retardation. Hypoxic fetoplacental vasoconstriction also occurs through the mechanism of potassium-channel inhibition.<sup>54</sup>

##### NEUROEPITHELIAL BODIES

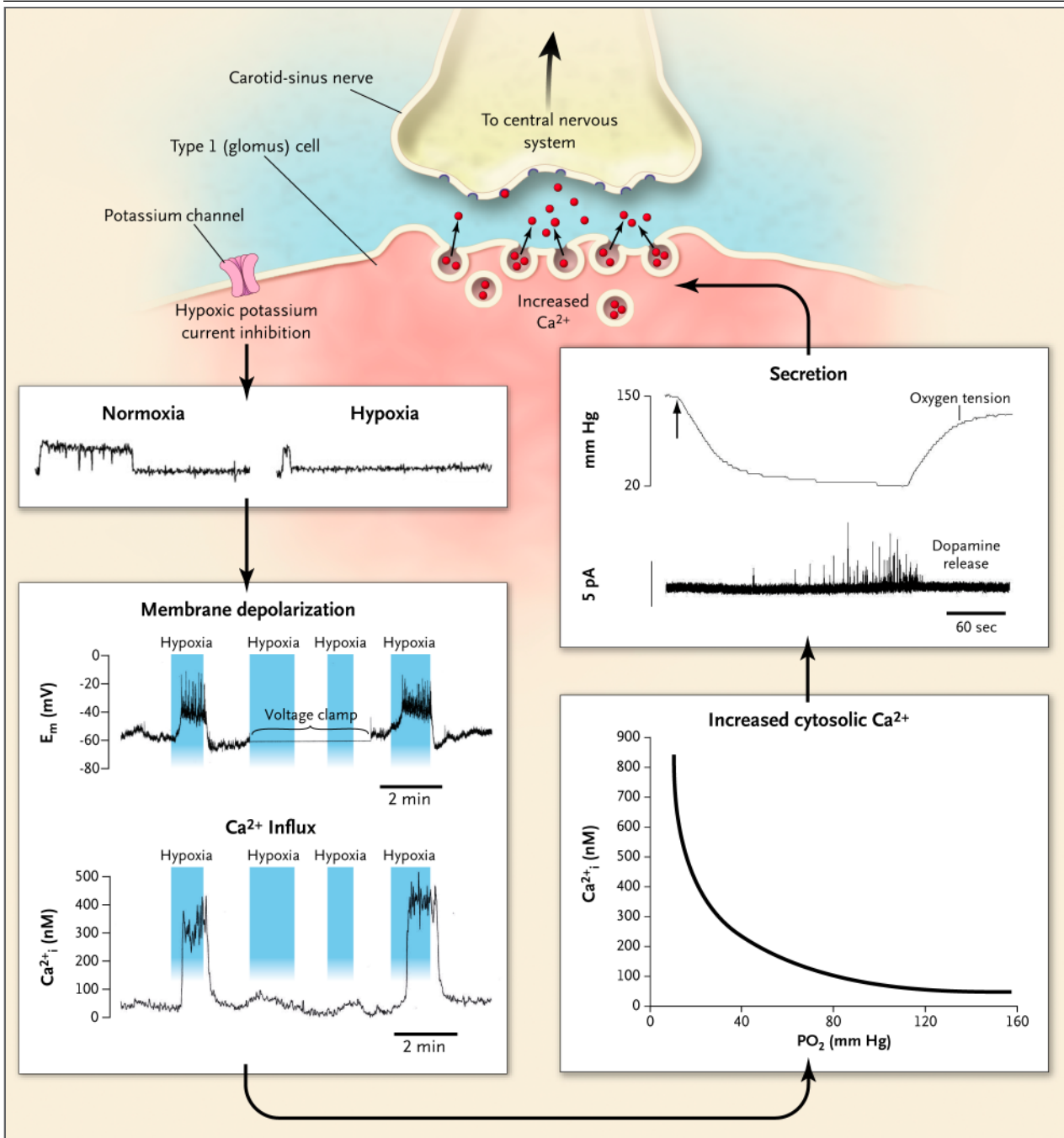
Neuroepithelial bodies are clusters of neuroendocrine cells in the mucosa of the airway that secrete amines and peptides in response to hypoxia.<sup>55</sup> They share a common origin with small-cell carcinoma of the lung and the derived cell line H146.<sup>56</sup> Neuroepithelial bodies may be involved in hypoxic pulmonary vasoconstriction and in respiratory control, especially in the neonate.<sup>57</sup> Hypoxia inhibits potassium current and causes membrane depolarization in neuroepithelial bodies and H146 cells,<sup>58,59</sup> leading to nifedipine-sensitive calcium influx<sup>57</sup> and secretion of serotonin, which has an important role in the vascular remodeling that occurs in chronic hypoxic pulmonary hypertension.

##### ADRENAL CHROMAFFIN CELLS

In the fetus and at the time of birth, the adrenal medulla responds to hypoxia by releasing catecholamines that improve fetal tolerance of labor. PC12 cells, derived from a pheochromocytoma, manifest hypoxic inhibition of potassium current, membrane depolarization, and an increase in cytosolic calcium,<sup>60</sup> which is largely inhibited by L-type calcium-channel blockers.

##### OTHER TISSUES

Oxygen-sensitive potassium channels have been reported in neurons,<sup>61</sup> in alveolar epithelial cells,<sup>62</sup> and in T lymphocytes<sup>63</sup>; these may be additional



**Figure 3. Oxygen Sensing in the Carotid Body.**

The chief function of the carotid body is to increase respiration in response to hypoxia. The proximal pathway in the type 1 cell of the carotid body is similar to that in the pulmonary-artery smooth-muscle cell. Hypoxia inhibits potassium-channel activity, shown in the single channel trace, causing membrane depolarization, calcium influx, secretion, and increased action potentials in the carotid-sinus nerve. If the membrane potential ( $E_m$ ) is "clamped" at  $-60$  mV, hypoxia no longer leads to an increase in the cytosolic calcium ( $Ca^{2+}_i$ ), indicating that the increase in calcium requires membrane depolarization. Cytosolic calcium normally rises sharply as oxygen levels fall below 60 mm Hg. Increased calcium stimulates the release of dopamine, a marker for secretion. pA denotes picoamperes.

tissue locations in which potassium channels mediate rapid responses (seconds to minutes) to altered oxygen tension. Other ion channels may also be regulated by changes in oxygen tension in muscle, neurons, and neurosecretory cells.<sup>64</sup>

---

#### OXYGEN SIGNALING

---

##### EFFECTS OF CHANGES IN THE REDUCTION–OXIDATION (REDOX) REACTION

Whereas the vasoconstrictors endothelin, phenylephrine, and potassium chloride constrict both the pulmonary artery and ductus arteriosus, hypoxia constricts the pulmonary artery but dilates the ductus arteriosus. This effect of hypoxia is shared only with certain agents that reduce sulfhydryl groups (redox agents) and inhibitors of complexes I and III of the mitochondrial electron-transport chain. The reducing agent dithiothreitol decreases potassium current, depolarizes membrane potential, increases cytosolic calcium, and constricts the pulmonary artery but has the opposite effects in the ductus arteriosus, which it relaxes, thus mimicking the effects of hypoxia.<sup>65</sup> Conversely, oxidizing agents mimic normoxia in both vessels. In the ductus arteriosus, where higher levels of oxygen tension (as at birth) increase reactive oxygen species, exogenous hydrogen peroxide mimics oxygen by inhibiting potassium current and depolarizing smooth-muscle cells of the ductus. Conversely, removal of endogenous hydrogen peroxide increases potassium current and hyperpolarizes these cells (Fig. 4).<sup>39,66</sup> Thus, changes in oxygen tension are signaled by changes in redox status. However, controversy exists, because some investigators report a decrease in reactive oxygen species during hypoxia, whereas others report an increase.<sup>11,67</sup>

##### SOURCES OF REACTIVE OXYGEN SPECIES

The probable sources of reactive oxygen species are NADPH and NADH oxidases or mitochondria, or both (Fig. 4). Genetic models provide evidence for<sup>68</sup> and against<sup>69,70</sup> a role for gp91phox-containing NADPH oxidase as an oxygen sensor. Potassium current is increased in neuroepithelial bodies, H146 cells, and adrenomedullary cells by hydrogen peroxide, suggesting that an increase in reactive oxygen species, such as hydrogen peroxide, mimics normoxia<sup>59,71</sup>; these observations accord with the view that hypoxia causes a reduction in reactive oxygen species. Similarly, both hypoxia and an inhibitor of NADPH oxidase in pulmonary-artery en-

dothelial cells reduce the release of hydrogen peroxide.<sup>72</sup> These studies may implicate NADPH oxidase in oxygen sensing. However, mitochondrial depletion prevents hypoxic contraction of single smooth-muscle cells in pulmonary arteries,<sup>73</sup> and mitochondrial inhibitors have opposite effects on potassium current and tone in the pulmonary artery and in the ductus arteriosus,<sup>39,74-76</sup> suggesting a role for mitochondria in the redox control of vascular tone. Inhibitors such as rotenone and antimycin (like hypoxia itself) alter the levels of redox signaling molecules, such as reactive oxygen species and the electron donors NADH and NADPH.<sup>76</sup>

##### MITOCHONDRIA AND OXYGEN SENSING IN THE CAROTID BODY

Inhibitors of mitochondrial function, such as rotenone, stimulate the carotid body,<sup>77</sup> suggesting that oxygen sensing is linked to energy metabolism. Many such inhibitors mimic the effects of hypoxia on glomus cells by causing inhibition of potassium current.<sup>78,79</sup> Moreover, background potassium channels, such as TASK, are sensitive to intracellular ATP, which is depleted by hypoxia.<sup>79</sup> Although these observations indicate that the carotid body is sensitive to changes in energy metabolism, it is uncertain whether these changes mediate oxygen sensing.<sup>80</sup> A novel hypothesis suggests that potassium current is maintained during normoxia by carbon monoxide generated by hemoxygenase-2.<sup>81</sup> The importance of this interesting concept will be easier to assess with membrane potential, calcium, and secretion data.

##### REDOX CHANGES AND CHANNEL GATING

Redox changes may alter potassium-channel gating and calcium release from the sarcoplasmic reticulum. The redox effect may directly affect the conductance of pore-forming  $\alpha$  subunits of potassium channels or could act through associated  $\beta$  subunits. For instance, cotransfection of Kv $\beta$ 1.3 with Kv1.5 into green monkey kidney cells (COS cells) inactivates Kv1.5 by facilitating NADPH binding to  $\beta$  subunits; this is reversed by the oxidized pyridine nucleotide NADP.<sup>82</sup> Hypoxia increases the ratio of reduced to oxidized redox pairs — NADPH–NADP and NADH–NAD — in the lung,<sup>83,84</sup> which would then inactivate the oxygen-sensitive potassium channels. A similar redox mechanism controls calcium release from the sarcoplasmic reticulum.<sup>85,86</sup> The hypoxic increase in NADH also increases cyclic ADP ribose, which promotes calcium release from

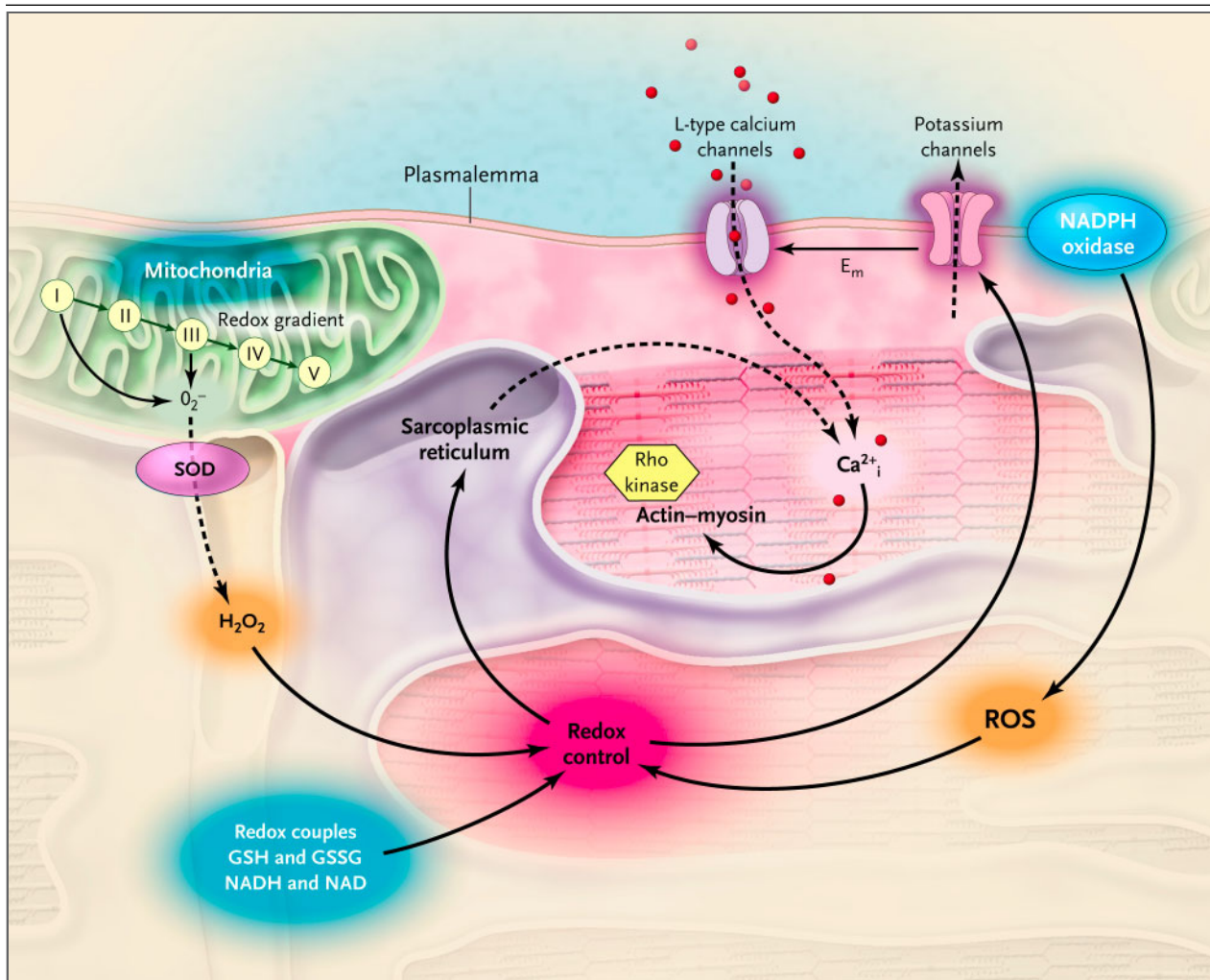


the sarcoplasmic reticulum of smooth-muscle cells of the pulmonary arteries<sup>87,88</sup> (Fig. 4). Thus, there is strong evidence linking changes in oxygen tension to changes in redox status and ultimately to potassium-channel and sarcoplasmic-reticulum gating. Although there is a concordance between low levels of reactive oxygen species and a reduced cytosolic–redox balance, it remains unclear whether one predominates as the signal linking the mitochondria or oxidase to potassium channels and sarcoplasmic reticulum.

CLINICAL SIGNIFICANCE

PULMONARY HYPERTENSION

The mechanisms of oxygen sensing relate to the treatment of pulmonary hypertension through identification of common pathways (control of membrane potential, cell proliferation, and apoptosis) and of probable therapeutic targets (Kv and store-operated channels, mitochondrial enzymes, the electron-transport chain, and Rho kinase). In the smooth-muscle cells of the pulmonary arteries from



**Figure 4. Redox Mechanism for Oxygen Sensing in Specialized Tissues.**

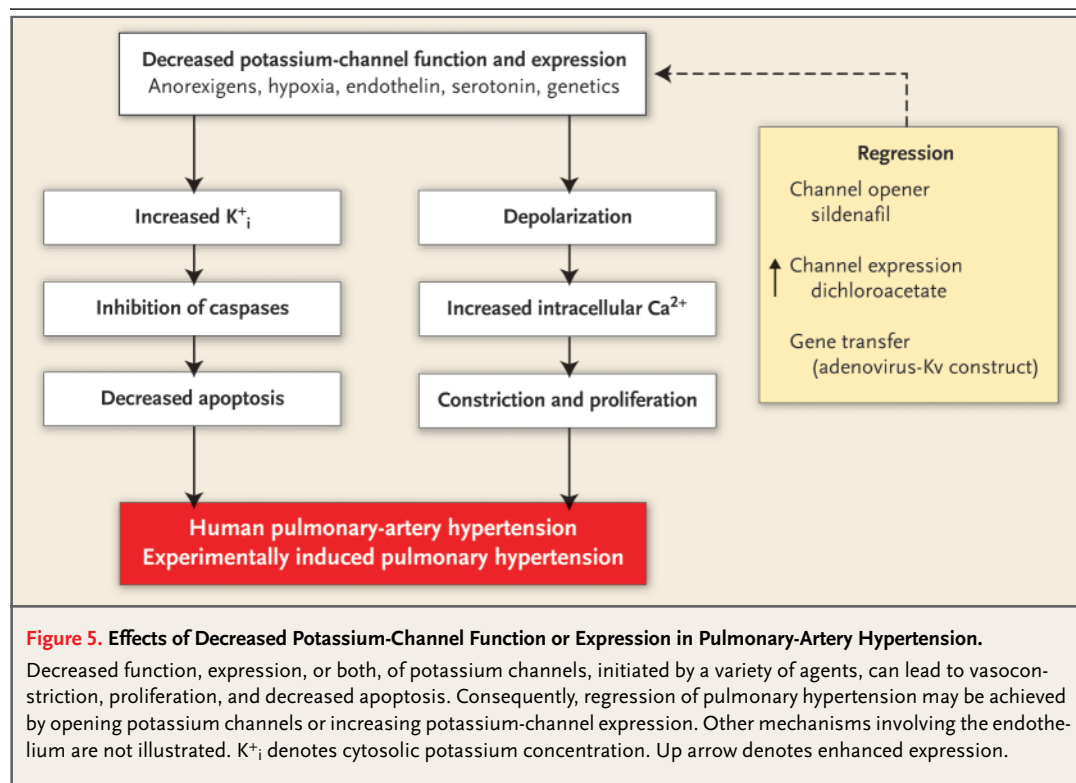
Reactive oxygen species (ROS) from the mitochondria, NADPH oxidase, NADH oxidase, or redox couples may control potassium-channel gating and membrane potential (E<sub>m</sub>) and thus calcium entry. The same redox signaling may control calcium release from the sarcoplasmic reticulum. The calcium stores in the sarcoplasmic reticulum, in turn, are repleted by calcium entry through the store-operated channels. Rho kinase augments the response of actin–myosin at any level of cytosolic calcium (Ca<sup>2+</sup><sub>i</sub>). SOD denotes superoxide dismutase, H<sub>2</sub>O<sub>2</sub> hydrogen peroxide, GSH glutathione, and GSSG oxidized glutathione.

patients with idiopathic pulmonary arterial hypertension, expression of specific potassium channels (e.g., Kv1.5 and Kv2.1) is reduced, membrane potential is depolarized, and cytosolic calcium is elevated, as compared with cells from patients with secondary pulmonary hypertension of similar severity.<sup>89</sup> This is related in part to abnormal bone morphogenetic protein-receptor signaling, which occurs in some patients with idiopathic pulmonary arterial hypertension.<sup>90</sup> The elevation of calcium promotes smooth-muscle-cell proliferation and hypertrophy in the pulmonary arteries. In addition, loss of potassium channels increases intracellular potassium, which inhibits apoptosis by blocking the activity of proapoptotic caspases.<sup>91</sup> Conversely, enhancing outward potassium current can initiate apoptosis, and this may be exploited therapeutically to cause regression of pulmonary hypertension<sup>17</sup> (Fig. 5).

The anorectic agent dexfenfluramine has been shown to increase the incidence of idiopathic pulmonary arterial hypertension.<sup>92</sup> Like hypoxia, anorectic agents inhibit potassium current in smooth-muscle cells of pulmonary arteries, block both Kv1.5 and Kv2.1, and release calcium from intra-

cellular stores.<sup>93,94</sup> Increased cytoplasmic calcium is an important signal for smooth-muscle-cell proliferation in the pulmonary arteries<sup>17</sup> (Fig. 5). Thus, there are close parallels between the mechanisms responsible for hypoxic pulmonary vasoconstriction and those involved in idiopathic pulmonary arterial hypertension and dexfenfluramine-related pulmonary hypertension. Conversely, drugs that enhance potassium current, such as sildenafil,<sup>95</sup> dichloroacetate,<sup>96</sup> and dehydroepiandrosterone,<sup>97</sup> may have a therapeutic benefit in pulmonary hypertension. In experimentally induced pulmonary hypertension, improvement has also been achieved with the use of aerosolized Kv1.5-channel gene therapy<sup>19</sup> (Fig. 5).

A role for store-operated calcium channels in idiopathic pulmonary arterial hypertension has been described recently,<sup>98</sup> and dihydropyridines, such as nifedipine, which block both L-type and store-operated calcium channels,<sup>99</sup> are effective in approximately 20 percent of these patients.<sup>100</sup> More specific blockers may prove more effective.<sup>28</sup> Rho kinase inhibitors reduce pulmonary hypertension induced experimentally by monocrotaline or chronic hypoxia.<sup>36,101</sup> If the delivery of Rho kinase inhib-



itors by inhalation prevents the deleterious effects of systemic vasodilatation,<sup>102</sup> they may prove helpful in treating pulmonary hypertension.

#### HIGH-ALTITUDE PULMONARY EDEMA

Hypoxic pulmonary vasoconstriction is strongest in small pulmonary arteries but occurs in pulmonary veins that also express various potassium channels that control their tone.<sup>103</sup> Given the inhibitory effects of hypoxia on potassium channels, it is not surprising that patients with high-altitude edema have increased hypoxic pulmonary vasoconstriction and higher pulmonary-capillary pressures.<sup>104</sup> Drugs that decrease calcium entry into pulmonary vascular smooth-muscle cells, such as nifedipine, may benefit these patients,<sup>105</sup> and sildenafil, which increases potassium current in smooth-muscle cells of the pulmonary arteries, improves exercise capacity at high altitude.<sup>106</sup>

#### DUCTUS ARTERIOSUS

Persistent patent ductus arteriosus is a common complication in markedly preterm infants, and prostaglandin H synthase inhibitors (e.g., indomethacin) frequently fail to close the ductus arteriosus, necessitating intervention.<sup>107</sup> Ex vivo transfer of the gene for Kv1.5 or Kv2.1 partially restores constriction to oxygen in the ductus arteriosus of the preterm rabbit. This may have therapeutic implications in persistent patent ductus arteriosus in preterm infants.

#### OXYGEN SENSING IN THE CAROTID BODY

Sensitivity of the carotid body to hypoxia increases during the early postnatal period, and this correlates with an increase in the magnitude of both normoxic potassium current and the hypoxic increase in cytosolic calcium. Loss of chemosensitivity due to carotid-body denervation shortly after the time of birth produces severe respiratory disturbances in several species, resulting in respiratory instability and unexpected death.<sup>108</sup> Similarly, patients with asthma who are treated with bilateral carotid-body ablation, a surgical procedure that is no longer in widespread use, have blunted responses to hypoxia, and some have died unexpectedly.<sup>109</sup> Carotid chemoreceptor denervation, which can occur with surgery of the neck, abolishes eupneic ventilatory responses to hypoxia and reduc-

es ventilatory responses to hypercapnia.<sup>110</sup> Sudden infant death syndrome could be due in part to changes in the carotid-body chemoreceptors.<sup>111</sup> Dopamine content in the carotid body is higher in affected children than in normal children,<sup>112</sup> and this higher level could cause carotid-body hyposensitivity, since dopamine is known to inhibit calcium current in glomus cells.<sup>113</sup> Interestingly, paragangliomas, which are tumors of the carotid body, can occur spontaneously in those living at high altitude, or the tumors can be inherited, because of mutations causing a loss of function in complex II of the mitochondria. These findings provide another suggestion that mitochondria are important in this specialized tissue.<sup>114,115</sup>

---

### CONCLUSIONS

---

The specialized tissues in the body that sense oxygen share a common mechanism that involves potassium channels, membrane potential, and L-type calcium channels. In vascular smooth-muscle cells, oxygen sensing also involves calcium release from the sarcoplasmic reticulum and calcium entry through store-operated channels, as well as calcium sensitization. Changes in cellular redox status may signal the alterations in oxygen, but whether reactive oxygen species are the key elements remains to be determined. Improved understanding of the mechanisms involved in the acute sensing of oxygen helps to explain the pathophysiology of idiopathic pulmonary arterial hypertension, persistent patent ductus arteriosus, and high-altitude pulmonary edema and provides insight into possible therapy.

Dr. Weir is supported by Veterans Affairs Merit Review Funding and a grant (ROI-HL-65322) from the National Heart, Lung, and Blood Institute. Dr. López-Barneo is supported by Fundación Juan March (Ayuda a la Investigación 2000), Fundación Lilly, the Spanish Ministry of Health and Andalusian Government. Dr. Buckler is supported by the British Heart Foundation and the Medical Research Council. Dr. Archer is supported by the Canada Foundation for Innovation, the Alberta Heart and Stroke Foundation, the Alberta Heritage Foundation for Medical Research, the Canadian Institutes for Health Research, a grant (RO1-HL071115) from the National Institutes of Health, and the Alberta Cardiovascular and Stroke Research Centre.

Dr. Archer reports holding a patent for the use of potassium-channel replacement therapy for the treatment of vascular diseases, including patent ductus arteriosus and pulmonary hypertension.

We acknowledge the lifelong leadership of John T. "Jack" Reeves, M.D. (1928–2004), in the field of oxygen sensing in the pulmonary vasculature.

## REFERENCES

1. Priestly J. Experiments and observations on different kinds of air. 2nd ed. London: J. Johnson, 1775:101.
2. Hales CA, Westphal D. Hypoxemia following the administration of sublingual nitroglycerin. *Am J Med* 1978;65:911-8.
3. Motley H, Cournard A, Werko L, Himmelstein A, Dresdale D. The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man. *Am J Physiol* 1947;150:315-20.
4. Hambraeus-Jonzon K, Bindsløv L, Mellgard A, Hedenstierna G. Hypoxic pulmonary vasoconstriction in human lungs: a stimulus-response study. *Anesthesiology* 1997;86:308-15.
5. Dorrington KL, Clar C, Young JD, Jonas M, Tansley JG, Robbins PA. Time course of the human pulmonary vascular response to 8 hours of isocapnic hypoxia. *Am J Physiol* 1997;273:H1126-H1134.
6. Madden JA, Vadula MS, Kurup VP. Effects of hypoxia and other vasoactive agents on pulmonary and cerebral artery smooth muscle cells. *Am J Physiol* 1992;263:L384-L393.
7. Post JM, Hume JR, Archer SL, Weir EK. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. *Am J Physiol* 1992;262:C882-C890.
8. Yuan X-J, Goldman WF, Tod ML, Rubin LJ, Blaustein MP. Hypoxia reduces potassium currents in cultured rat pulmonary but not mesenteric arterial myocytes. *Am J Physiol* 1993;264:L116-L123.
9. Olschewski A, Hong Z, Nelson DP, Weir EK. Graded response of K<sup>+</sup> current, membrane potential, and [Ca<sup>2+</sup>]<sub>i</sub> to hypoxia in pulmonary arterial smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1143-L1150.
10. Archer SL, Huang JM, Reeve HL, et al. The differential distribution of electrophysiologically distinct myocytes in conduit and resistance arteries determines their response to nitric oxide and hypoxia. *Circ Res* 1996;78:431-42.
11. Michelakis ED, Thebaud B, Weir EK, Archer SL. Hypoxic pulmonary vasoconstriction: redox regulation of O<sub>2</sub>-sensitive K<sup>(+)</sup> channels by a mitochondrial O<sub>2</sub>-sensor in resistance artery smooth muscle cells. *J Mol Cell Cardiol* 2004;37:1119-36.
12. Cornfield DN, Reeve HL, Tolarova S, Weir EK, Archer S. Oxygen causes fetal pulmonary vasodilation through activation of a calcium-dependent potassium channel. *Proc Natl Acad Sci U S A* 1996;93:8089-94.
13. Archer SL, Wu XC, Thebaud B, et al. Preferential expression and function of voltage-gated, O<sub>2</sub>-sensitive K<sup>+</sup> channels in resistance pulmonary arteries explains regional heterogeneity in hypoxic pulmonary vasoconstriction: ionic diversity in smooth muscle cells. *Circ Res* 2004;95:308-18.
14. McMurtry IF, Petrun MD, Reeves JT. Lungs from chronically hypoxic rats have decreased pressor response to acute hypoxia. *Am J Physiol* 1978;235:H104-H109.
15. Smirnov SV, Robertson TP, Ward JP, Aaronson PI. Chronic hypoxia is associated with reduced delayed rectifier K<sup>+</sup> current in rat pulmonary artery muscle cells. *Am J Physiol* 1994;266:H365-H370.
16. Osipenko ON, Alexander D, MacLean MR, Gurney AM. Influence of chronic hypoxia on the contributions of non-inactivating and delayed rectifier K currents to the resting potential and tone of rat pulmonary artery smooth muscle. *Br J Pharmacol* 1998;124:1335-7.
17. Krick S, Platoshyn O, McDaniel SS, Rubin LJ, Yuan JX. Augmented K<sup>(+)</sup> currents and mitochondrial membrane depolarization in pulmonary artery myocyte apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L887-L894.
18. Reeve HL, Michelakis E, Nelson DP, Weir EK, Archer SL. Alterations in a redox oxygen sensing mechanism in chronic hypoxia. *J Appl Physiol* 2001;90:2249-56.
19. Pozeg ZI, Michelakis ED, McMurtry MS, et al. In vivo gene transfer of the O<sub>2</sub>-sensitive potassium channel Kv1.5 reduces pulmonary hypertension and restores hypoxic pulmonary vasoconstriction in chronically hypoxic rats. *Circulation* 2003;107:2037-44.
20. Osipenko ON, Tate RJ, Gurney AM. Potential role for Kv3.1b channels as oxygen sensors. *Circ Res* 2000;86:534-40.
21. Hogg DS, Davies AR, McMurray G, Kozlowski RZ. K(V)2.1 channels mediate hypoxic inhibition of I<sub>(KV)</sub> in native pulmonary arterial smooth muscle cells of the rat. *Cardiovasc Res* 2002;55:349-60.
22. Robertson TP, Hague D, Aaronson PI, Ward JP. Voltage-independent calcium entry in hypoxic pulmonary vasoconstriction of intrapulmonary arteries of the rat. *J Physiol* 2000;525:669-80.
23. Burghuber OC. Nifedipine attenuates acute hypoxic pulmonary vasoconstriction in patients with chronic obstructive pulmonary disease. *Respiration* 1987;52:86-93.
24. Salvaterra CG, Goldman WF. Acute hypoxia increases cytosolic calcium in cultured pulmonary arterial myocytes. *Am J Physiol* 1993;264:L323-L328.
25. Dipp M, Nye PC, Evans AM. Hypoxic release of calcium from the sarcoplasmic reticulum of pulmonary artery smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L318-L325.
26. Morio Y, McMurtry IF. Ca<sup>2+</sup> release from ryanodine-sensitive store contributes to mechanism of hypoxic vasoconstriction in rat lungs. *J Appl Physiol* 2002;92:527-34.
27. Wang J, Shimoda LA, Weigand L, Wang W, Sun D, Sylvester JT. Acute hypoxia increases intracellular [Ca<sup>2+</sup>]<sub>i</sub> in pulmonary arterial smooth muscle by enhancing capacitative Ca<sup>2+</sup> entry. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L1059-L1069.
28. Weigand L, Foxson J, Wang J, Shimoda LA, Sylvester JT. Inhibition of hypoxic pulmonary vasoconstriction by store-operated Ca<sup>2+</sup> and nonselective cation channel antagonists. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L5-L13.
29. Ng LC, Wilson SM, Hume JR. Mobilization of sarcoplasmic reticulum stores by hypoxia leads to consequent activation of capacitative Ca<sup>2+</sup> entry in isolated canine pulmonary arterial smooth muscle cells. *J Physiol* 2005;563:409-19.
30. Landsberg JW, Yuan J-X. Calcium and TRP channels in pulmonary vascular smooth muscle cell proliferation. *News Physiol Sci* 2004;19:44-50.
31. Somlyo AP, Somlyo AV. Ca<sup>2+</sup> sensitivity of smooth muscle and non-muscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003;83:1325-58.
32. Robertson TP, Aaronson PI, Ward JP. Hypoxic vasoconstriction and intracellular Ca<sup>2+</sup> in pulmonary arteries: evidence for PKC-independent Ca<sup>2+</sup> sensitization. *Am J Physiol* 1995;268:H301-H307.
33. Wang Z, Jin N, Ganguli S, Swartz DR, Li L, Rhoades RA. Rho-kinase activation is involved in hypoxia-induced pulmonary vasoconstriction. *Am J Respir Cell Mol Biol* 2001;25:628-35.
34. Ward JP, Knock GA, Snetkov VA, Aaronson PI. Protein kinases in vascular smooth muscle tone — role in the pulmonary vasculature and hypoxic pulmonary vasoconstriction. *Pharmacol Ther* 2004;104:207-31.
35. Robertson TP, Dipp M, Ward JP, Aaronson PI, Evans AM. Inhibition of sustained hypoxic vasoconstriction by Y-27632 in isolated intrapulmonary arteries and perfused lung of the rat. *Br J Pharmacol* 2000;131:5-9.
36. Fagan KA, Oka M, Bauer NR, et al. Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L656-L664.
37. Alzamora-Castro V, Battilana G, Abigatas R, Sialer S. Patent ductus arteriosus and high altitude. *Am J Cardiol* 1960;5:761-3.
38. Tristani-Firouzi M, Reeve HL, Tolarova S, Weir EK, Archer SL. Oxygen-induced constriction of rabbit ductus arteriosus occurs via inhibition of a 4-aminopyridine-, voltage-sensitive potassium channel. *J Clin Invest* 1996;98:1959-65.
39. Michelakis ED, Rebecka I, Wu X, et al. O<sub>2</sub> sensing in the human ductus arteriosus: regulation of voltage-gated K<sup>+</sup> channels in smooth muscle cells by a mitochondrial redox sensor. *Circ Res* 2002;91:478-86.
40. Michelakis E, Rebecka I, Bateson J, Olley P, Puttagunta L, Archer S. Voltage-gated potassium channels in human ductus arteriosus. *Lancet* 2000;356:134-7.
41. Lopez-Barneo J, Lopez-Lopez JR, Urena J, Gonzalez C. Chemotransduction in the

- carotid body: K<sup>+</sup> current modulated by PO<sub>2</sub> in type I chemoreceptor cells. *Science* 1988; 241:580-2.
42. Ganformina MD, Lopez-Barneo J. Potassium channel types in arterial chemoreceptor cells and their selective modulation by oxygen. *J Gen Physiol* 1992;100:401-26.
43. Wyatt CN, Wright C, Bee D, Peers C. O<sub>2</sub>-sensitive K<sup>+</sup> currents in carotid body chemoreceptor cells from normoxic and chronically hypoxic rats and their role in hypoxic chemotransduction. *Proc Natl Acad Sci U S A* 1995;92:295-9.
44. Buckler KJ. A novel oxygen-sensitive potassium current in rat carotid body type I cells. *J Physiol* 1997;498:649-62.
45. Buckler KJ, Williams BA, Honore E. An oxygen-, acid- and anaesthetic-sensitive TASK-like background potassium channel in rat arterial chemoreceptor cells. *J Physiol* 2000;525:135-42.
46. Buckler KJ, Vaughan-Jones RD. Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells. *J Physiol* 1994;476:423-8.
47. Urena J, Fernandez-Chacon R, Benot AR, Alvarez de Toledo GA, Lopez-Barneo J. Hypoxia induces voltage-dependent Ca<sup>2+</sup> entry and quantal dopamine secretion in carotid body glomus cells. *Proc Natl Acad Sci U S A* 1994;91:10208-11.
48. Montoro RJ, Urena J, Fernandez-Chacon R, Alvarez de Toledo G, Lopez-Barneo J. Oxygen sensing by ion channels and chemotransduction in single glomus cells. *J Gen Physiol* 1996;107:133-43.
49. Pardal R, Ludewig U, Garcia-Hirschfeld J, Lopez-Barneo J. Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium. *Proc Natl Acad Sci U S A* 2000;97:2361-6.
50. Peers C. Effects of doxapram on ionic currents recorded in isolated type I cells of the neonatal rat carotid body. *Brain Res* 1991;568:116-22.
51. Severinghaus JW, Bainton CR, Carcelen A. Respiratory insensitivity to hypoxia in chronically hypoxic man. *Respir Physiol* 1966;1:308-34.
52. Shimoda LA, Manalo DJ, Sham JS, Semenza GL, Sylvester JT. Partial HIF-1 $\alpha$  deficiency impairs pulmonary arterial myocyte electrophysiological responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L202-L208.
53. Howard RB, Hosokawa T, Maguire HH. Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons. *Am J Obstet Gynecol* 1987;157:1261-6.
54. Hampl V, Bibova J, Stranek Z, et al. Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition. *Am J Physiol Heart Circ Physiol* 2002;283:H2440-H2449.
55. Lauweryns JM, Cokelaere M, Deleersnyder M, Liebens M. Intrapulmonary neuroepithelial bodies in newborn rabbits: influence of hypoxia, hyperoxia, hypercapnia, nicotine, reserpine, L-DOPA and 5-HTP. *Cell Tissue Res* 1977;182:425-40.
56. Kemp PJ, Lewis A, Hartness ME, et al. Airway chemotransduction: from oxygen sensor to cellular effector. *Am J Respir Crit Care Med* 2002;166:S17-S24.
57. Fu XW, Nurse CA, Wong V, Cutz E. Hypoxia-induced secretion of serotonin from intact pulmonary neuroepithelial bodies in neonatal rabbit. *J Physiol* 2002;539:503-10.
58. Youngson C, Nurse C, Yeger H, Cutz E. Oxygen sensing in airway chemoreceptors. *Nature* 1993;365:153-5.
59. O'Kelly I, Lewis A, Peers C, Kemp PJ. O(2) sensing by airway chemoreceptor-derived cells: protein kinase C activation reveals functional evidence for involvement of NADPH oxidase. *J Biol Chem* 2000;275:7684-92.
60. Zhu WH, Conforti L, Czyzyk-Krzeska MF, Milhorn DE. Membrane depolarization in PC-12 cells during hypoxia is regulated by an O<sub>2</sub>-sensitive K<sup>+</sup> current. *Am J Physiol* 1996;271:C658-C665.
61. Jiang C, Haddad GG. Oxygen deprivation inhibits a K<sup>+</sup> channel independently of cytosolic factors in rat central neurons. *J Physiol* 1994;481:15-26.
62. Jovanovic S, Crawford RM, Ranki HJ, Jovanovic A. Large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels sense acute changes in oxygen tension in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 2003;28:363-72.
63. Conforti L, Petrovic M, Mohammad D, et al. Hypoxia regulates expression and activity of Kv1.3 channels in T lymphocytes: a possible role in T cell proliferation. *J Immunol* 2003;170:695-702.
64. Lopez-Barneo J, Pardal R, Ortega-Saenz P. Cellular mechanism of oxygen sensing. *Annu Rev Physiol* 2001;63:259-87.
65. Olschewski A, Hong Z, Peterson DA, Nelson DP, Porter VA, Weir EK. Opposite effects of redox status on membrane potential, cytosolic calcium, and tone in pulmonary arteries and ductus arteriosus. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L15-L22.
66. Reeve H, Tolarova S, Nelson DP, Archer S, Weir EK. Redox control of oxygen sensing in the rabbit ductus arteriosus. *J Physiol* 2001;533:253-61.
67. Waypa GB, Schumacker PT. Hypoxic pulmonary vasoconstriction: redox events in oxygen sensing. *J Appl Physiol* 2005;98:404-14.
68. Fu XW, Wang D, Nurse CA, Dinauer MC, Cutz E. NADPH oxidase is an O<sub>2</sub> sensor in airway chemoreceptors: evidence from K<sup>+</sup> current modulation in wild-type and oxidase-deficient mice. *Proc Natl Acad Sci U S A* 2000;97:4374-9.
69. Archer SL, Reeve HL, Michelakis E, et al. O<sub>2</sub> sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. *Proc Natl Acad Sci U S A* 1999;96:7944-9.
70. Roy A, Rozanov C, Mokashi A, et al. Mice lacking in gp91 phox subunit of NAD(P)H oxidase showed glomus cell [Ca(2+)](i) and respiratory responses to hypoxia. *Brain Res* 2000;872:188-93.
71. Wang D, Youngson C, Wong V, et al. NADPH-oxidase and a hydrogen peroxide-sensitive K<sup>+</sup> channel may function as an oxygen sensor complex in airway chemoreceptors and small cell lung carcinoma cell lines. *Proc Natl Acad Sci U S A* 1996;93:13182-7.
72. Zulueta JJ, Yu FS, Hertig IA, Thannickal VJ, Hassoun PM. Release of hydrogen peroxide in response to hypoxia-reoxygenation: role of an NAD(P)H oxidase-like enzyme in endothelial cell plasma membrane. *Am J Respir Cell Mol Biol* 1995;12:41-9.
73. Waypa GB, Chandel NS, Schumacker PT. Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. *Circ Res* 2001;88:1259-66.
74. Archer SL, Huang J, Henry T, Peterson D, Weir EK. A redox-based O<sub>2</sub> sensor in rat pulmonary vasculature. *Circ Res* 1993;73:1100-12.
75. Archer SL, Wu XC, Thebaud B, Moudgil R, Hashimoto K, Michelakis ED. O<sub>2</sub> sensing in the human ductus arteriosus: redox-sensitive K<sup>+</sup> channels are regulated by mitochondria-derived hydrogen peroxide. *Biol Chem* 2004;385:205-16.
76. Michelakis ED, Hampl V, Nsair A, et al. Diversity in mitochondrial function explains differences in vascular oxygen sensing. *Circ Res* 2002;90:1307-15.
77. Anichcov SV, Belen'kii ML. Pharmacology of the carotid body chemoreceptors. Crawford R, trans. New York: Macmillan, 1963.
78. Wyatt CN, Buckler KJ. The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. *J Physiol* 2004;556:175-91.
79. Williams BA, Buckler KJ. Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type 1 cells. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L221-L230.
80. Ortega-Saenz P, Pardal R, Garcia-Fernandez M, Lopez-Barneo J. Rotenone selectively occludes sensitivity to hypoxia in rat carotid body glomus cells. *J Physiol* 2003; 548:789-800.
81. Williams SE, Wootton P, Mason HS, et al. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. *Science* 2004;306:2093-7.
82. Tipparaju SM, Saxena N, Liu SQ, Kumar R, Bhatnagar A. Differential regulation of voltage-gated K<sup>+</sup> channels by oxidized and reduced pyridine nucleotide coenzymes. *Am J Physiol Cell Physiol* 2005;288:C366-C376.
83. Chander A, Dhariwal KR, Viswanathan R, Venkatasubramanian TA. Pyridine nucleotides in lung and liver of hypoxic rats. *Life Sci* 1980;26:1935-45.
84. Leach RM, Hill HM, Snetkov VA, Robertson TP, Ward JP. Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor. *J Physiol* 2001;536:211-24.
85. Kaplin AI, Snyder SH, Linden DJ. Reduced nicotinamide adenine dinucleotide-selective stimulation of inositol 1,4,5-trisphosphate receptors mediates hypoxic

- mobilization of calcium. *J Neurosci* 1996; 16:2002-11.
86. Cherednichenko G, Zima AV, Feng W, Schaefer S, Blatter LA, Pessah IN. NADH oxidase activity of rat cardiac sarcoplasmic reticulum regulates calcium-induced calcium release. *Circ Res* 2004;94:478-86.
87. Wilson HL, Dipp M, Thomas JM, Lad C, Galione A, Evans AM. ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase act as a redox sensor: a primary role for cyclic ADP-ribose in hypoxic pulmonary vasoconstriction. *J Biol Chem* 2001;276:11180-8.
88. Dipp M, Evans AM. Cyclic ADP-ribose is the primary trigger for hypoxic pulmonary vasoconstriction in the rat lung in situ. *Circ Res* 2001;89:77-83.
89. Yuan JX, Aldinger AM, Juhaszova M, et al. Dysfunctional voltage-gated K<sup>+</sup> channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. *Circulation* 1998;98:1400-6.
90. Remillard C, Fantozzi I, Platoshyn O, et al. Bone morphogenetic protein-2 enhances K<sub>v</sub> channel expression and function in human pulmonary artery smooth muscle cells. *Proc Am Thorac Soc* 2005;2:A723. abstract.
91. Hughes FM Jr, Bortner CD, Purdy GD, Cidlowski JA. Intracellular K<sup>+</sup> suppresses the activation of apoptosis in lymphocytes. *J Biol Chem* 1997;272:30567-76.
92. Abenhaim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *N Engl J Med* 1996;335:609-16.
93. Weir EK, Reeve HL, Huang JM, et al. Anorexic agents aminorex, fenfluramine, and dexfenfluramine inhibit potassium current in rat pulmonary vascular smooth muscle and cause pulmonary vasoconstriction. *Circulation* 1996;94:2216-20.
94. Hong Z, Olschewski A, Reeve HL, Nelson DP, Hong F, Weir EK. Nordexfenfluramine causes more severe pulmonary vasoconstriction than dexfenfluramine. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L531-L538.
95. Michelakis ED, Tymchak W, Noga M, et al. Long-term treatment with oral sildenafil is safe and improves functional capacity and hemodynamics in patients with pulmonary arterial hypertension. *Circulation* 2003;108:2066-9.
96. McMurry M, Bonnet S, Wu X, et al. Dichloroacetate prevents and reverses pulmonary hypertension by inducing pulmonary artery smooth muscle cell apoptosis. *Circ Res* 2004;95:830-40.
97. Peng W, Hoidal JR, Farrukh IS. Role of a novel KCa opener in regulating K<sup>+</sup> channels of hypoxic human pulmonary vascular cells. *Am J Respir Cell Mol Biol* 1999;20:737-45.
98. Yu Y, Fantozzi I, Remillard CV, et al. Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc Natl Acad Sci U S A* 2004;101:13861-6.
99. Curtis TM, Scholfield CN. Nifedipine blocks Ca<sup>2+</sup> store refilling through a pathway not involving L-type Ca<sup>2+</sup> channels in rabbit arteriolar smooth muscle. *J Physiol* 2001;532:609-23.
100. Rich S, Brundage BH. High-dose calcium channel-blocking therapy for primary pulmonary hypertension: evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. *Circulation* 1987;76:135-41.
101. Abe K, Shimokawa H, Morikawa K, et al. Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. *Circ Res* 2004;94:385-93.
102. Nagaoka T, Fagan KA, Gebb SA, et al. Inhaled Rho kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension. *Am J Respir Crit Care Med* 2005;171:494-9.
103. Michelakis ED, Weir EK, Wu X, et al. Potassium channels regulate tone in rat pulmonary veins. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1138-L1147.
104. Maggiorini M, Melot C, Pierre S, et al. High-altitude pulmonary edema is initially caused by an increase in capillary pressure. *Circulation* 2001;103:2078-83.
105. Bartsch P, Mairbaurl H, Swenson ER, Maggiorini M. High altitude pulmonary oedema. *Swiss Med Wkly* 2003;133:377-84.
106. Ghofrani HA, Reichenberger F, Kohstall MG, et al. Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. *Ann Intern Med* 2004;141:169-77.
107. Casalez D. Ibuprofen versus indomethacin for closure of patent ductus arteriosus. *N Engl J Med* 2001;344:457-8.
108. Donnelly DF. Developmental aspects of oxygen sensing by the carotid body. *J Appl Physiol* 2000;88:2296-301.
109. Sullivan CE. Bilateral carotid body resection in asthma: vulnerability to hypoxic death in sleep. *Chest* 1980;78:354.
110. Timmers HJ, Wieling W, Karemaker MJ, Lenders JW. Denervation of carotid baro- and chemoreceptors in humans. *J Physiol* 2003;553:3-11.
111. Gauda EB, McLemore GL, Tolosa J, Marston-Nelson J, Kwak D. Maturation of peripheral arterial chemoreceptors in relation to neonatal apnoea. *Semin Neonatol* 2004;9:181-94.
112. Perrin DG, Cutz E, Becker LE, Bryan AC, Madapallimatum A, Sole MJ. Sudden infant death syndrome: increased carotid-body dopamine and noradrenaline content. *Lancet* 1984;2:535-7.
113. Benot AR, Lopez-Barneo J. Feedback inhibition of Ca<sup>2+</sup> currents by dopamine in glomus cells of the carotid body. *Eur J Neurosci* 1990;2:809-12.
114. Baysal BE, Myers EN. Etiopathogenesis and clinical presentation of carotid body tumors. *Microsc Res Tech* 2002;59:256-61.
115. Piruat JI, Pintado CO, Ortega-Saenz P, Roche M, Lopez-Barneo J. The mitochondrial SDHD gene is required for early embryogenesis and its partial deficiency results in persistent carotid body glomus cells activation with full responsiveness to hypoxia. *Mol Cell Biol* 2004;24:10933-40.

Copyright © 2005 Massachusetts Medical Society.

FULL TEXT OF ALL JOURNAL ARTICLES ON THE WORLD WIDE WEB

Access to the complete text of the *Journal* on the Internet is free to all subscribers. To use this Web site, subscribers should go to the *Journal's* home page ([www.nejm.org](http://www.nejm.org)) and register by entering their names and subscriber numbers as they appear on their mailing labels. After this one-time registration, subscribers can use their passwords to log on for electronic access to the entire *Journal* from any computer that is connected to the Internet. Features include a library of all issues since January 1993 and abstracts since January 1975, a full-text search capacity, and a personal archive for saving articles and search results of interest. All articles can be printed in a format that is virtually identical to that of the typeset pages. Beginning six months after publication, the full text of all Original Articles and Special Articles is available free to nonsubscribers who have completed a brief registration.