

REVIEW ARTICLE

MECHANISMS OF DISEASE

Acute Oxygen-Sensing Mechanisms

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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.”¹

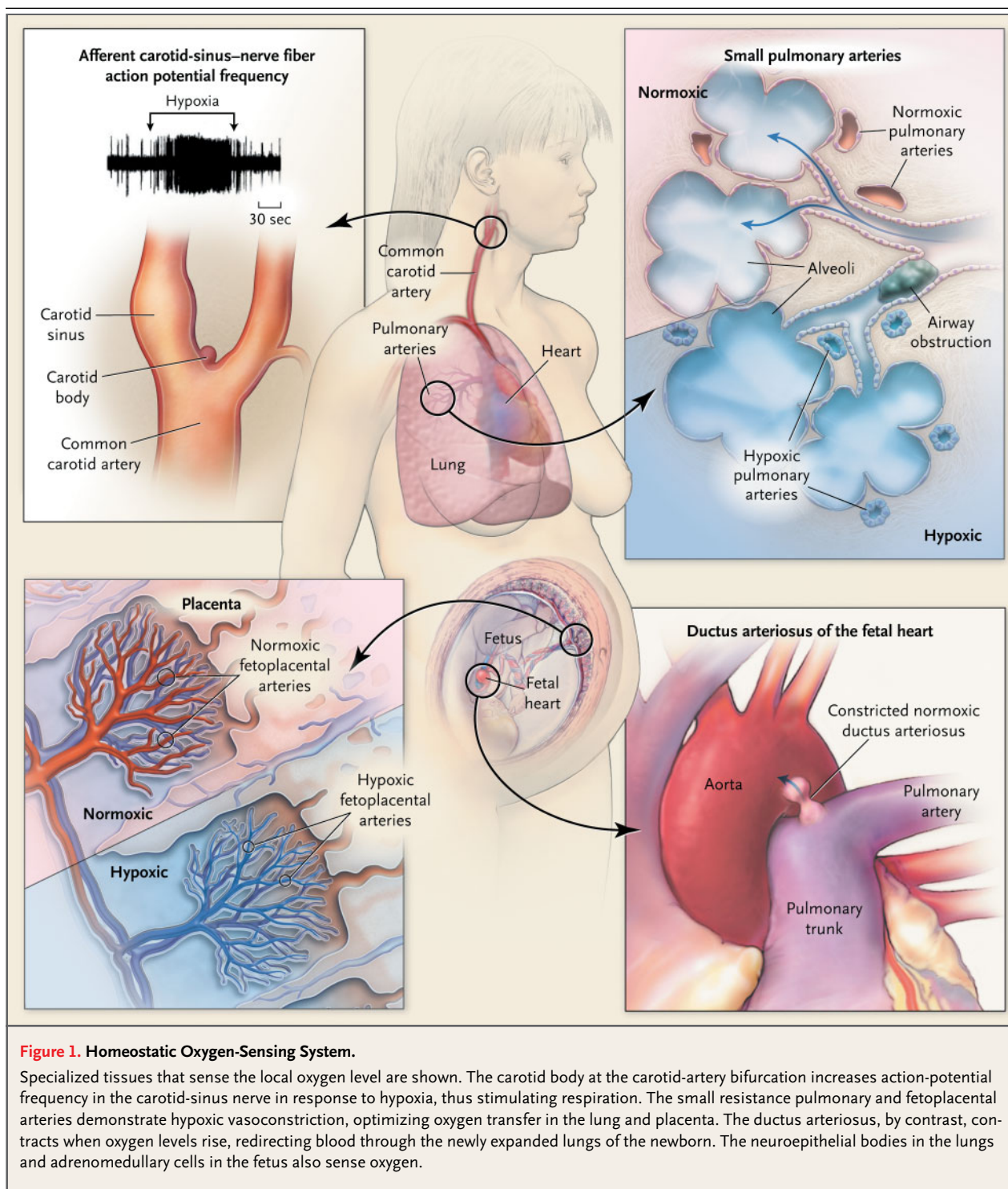
In this review we examine the remarkable mechanisms by which different organs detect and respond to acute changes in oxygen tension. Specialized tissues that sense the local oxygen tension include glomus cells of the carotid body, neuroepithelial bodies in the lungs, chromaffin cells of the fetal adrenal 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.² Moreover, as was first demonstrated in humans in 1947,³ the intensity of hypoxic pulmonary vasoconstriction depends on the severity and duration of alveolar hypoxia.^{4,5}

The endothelium produces vasodilators, such as nitric oxide and prostacyclin, and vasoconstrictors, such as endothelin and thromboxane A₂; 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.⁶ 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).^{7,8} 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. β 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.^{7,8} 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 hypoxia⁹ and is more prominent in small resistance pulmonary arteries (diameter, <500 μ m) than in large extraparenchymal pulmonary arteries.¹⁰

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

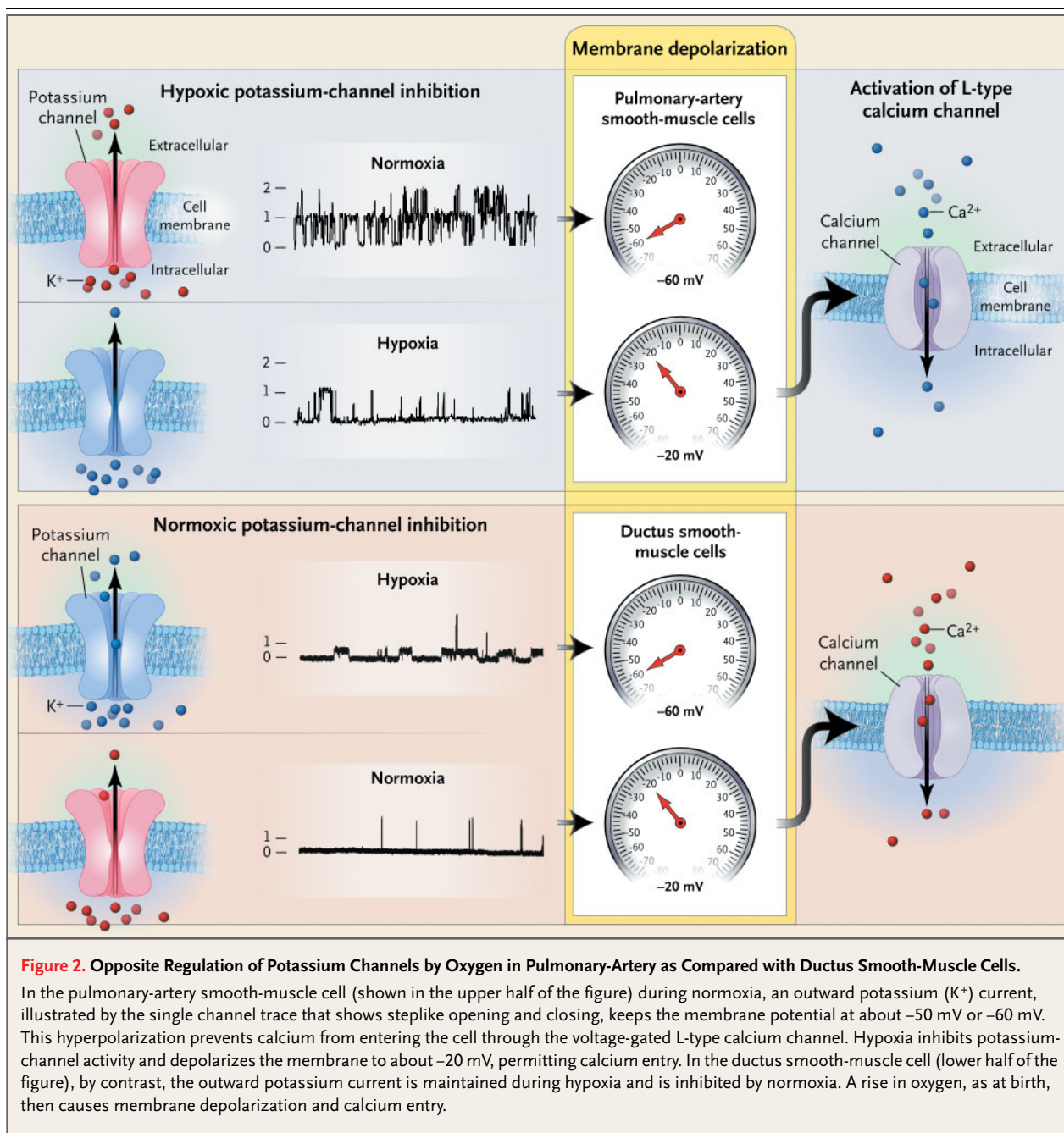


Figure 2. Opposite Regulation of Potassium Channels by Oxygen in Pulmonary-Artery as Compared with Ductus Smooth-Muscle Cells.

In the pulmonary-artery smooth-muscle cell (shown in the upper half of the figure) during normoxia, an outward potassium (K⁺) current, illustrated by the single channel trace that shows steplike opening and closing, keeps the membrane potential at about -50 mV or -60 mV. This hyperpolarization prevents calcium from entering the cell through the voltage-gated L-type calcium channel. Hypoxia inhibits potassium-channel activity and depolarizes the membrane to about -20 mV, permitting calcium entry. In the ductus smooth-muscle cell (lower half of the figure), by contrast, the outward potassium current is maintained during hypoxia and is inhibited by normoxia. A rise in oxygen, as at birth, then causes membrane depolarization and calcium entry.

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

blunted in rats previously exposed to chronic hypoxia,¹⁴ 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.¹⁵⁻¹⁸ 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_v1.5,¹⁹ 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.^{13,20,21} 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.²² 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.²³ Whereas most of the calcium involved in hypoxic pulmonary vasoconstriction comes from outside the cell, some comes from internal stores.⁹ 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.²⁴⁻²⁶ 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.²⁷⁻³⁰ Inhibitors of these channels block calcium influx into the smooth-muscle cells of the pulmonary arteries that are stimulated by hypoxia²⁷ and prevent hypoxic pulmonary vasoconstriction.²⁸

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.³¹ 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.³² Hypoxia, acting through the small G protein RhoA, stimulates Rho kinase,³³ which inhibits myosin light-chain phosphatase, thereby increasing phosphorylation of the light chain and augmenting contraction. Consequently, Rho kinase increases hypoxic pulmonary vasoconstriction,³⁴ and Rho-kinase inhibitors reduce hypoxic pulmonary vasoconstriction.^{35,36}

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.³⁷ Oxygen-induced membrane depolarization in the ductus arteriosus occurs because an increase in oxygen tension inhibits Kv channels (Fig. 2).^{38,39} 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.^{38,40}

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.³⁹ 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.⁴¹ Subsequently, Kv, K_{Ca}, and TASK-like glomus-cell potassium channels were shown to be oxygen-sensitive.⁴²⁻⁴⁵ 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).^{46,47} 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.⁴⁶ The increase in cytosolic calcium is closely related to the secretion of dopamine (a neurotransmitter abundant in glomus cells) (Fig. 3),⁴⁸ and hypoxia-induced neurosecretion is prevented by removal of extracellular calcium.⁴⁹

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_{Ca} channels, cause neurosecretion in cultured slices of the carotid body of rats also indicates that K_{Ca} 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_{Ca} and Kv currents in glomus cells.⁵⁰

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.⁴³ 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.⁵¹ In the pulmonary artery, the decreased expression of potassium channels is in part signaled by hypoxia-inducible factor 1 α (usually referred to as HIF-1 α),⁵² 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.^{53,54} 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.⁵⁴

NEUROEPITHELIAL BODIES

Neuroepithelial bodies are clusters of neuroendocrine cells in the mucosa of the airway that secrete amines and peptides in response to hypoxia.⁵⁵ They share a common origin with small-cell carcinoma of the lung and the derived cell line H146.⁵⁶ Neuroepithelial bodies may be involved in hypoxic pulmonary vasoconstriction and in respiratory control, especially in the neonate.⁵⁷ Hypoxia inhibits potassium current and causes membrane depolarization in neuroepithelial bodies and H146 cells,^{58,59} leading to nifedipine-sensitive calcium influx⁵⁷ 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,⁶⁰ which is largely inhibited by L-type calcium-channel blockers.

OTHER TISSUES

Oxygen-sensitive potassium channels have been reported in neurons,⁶¹ in alveolar epithelial cells,⁶² and in T lymphocytes⁶³; these may be additional

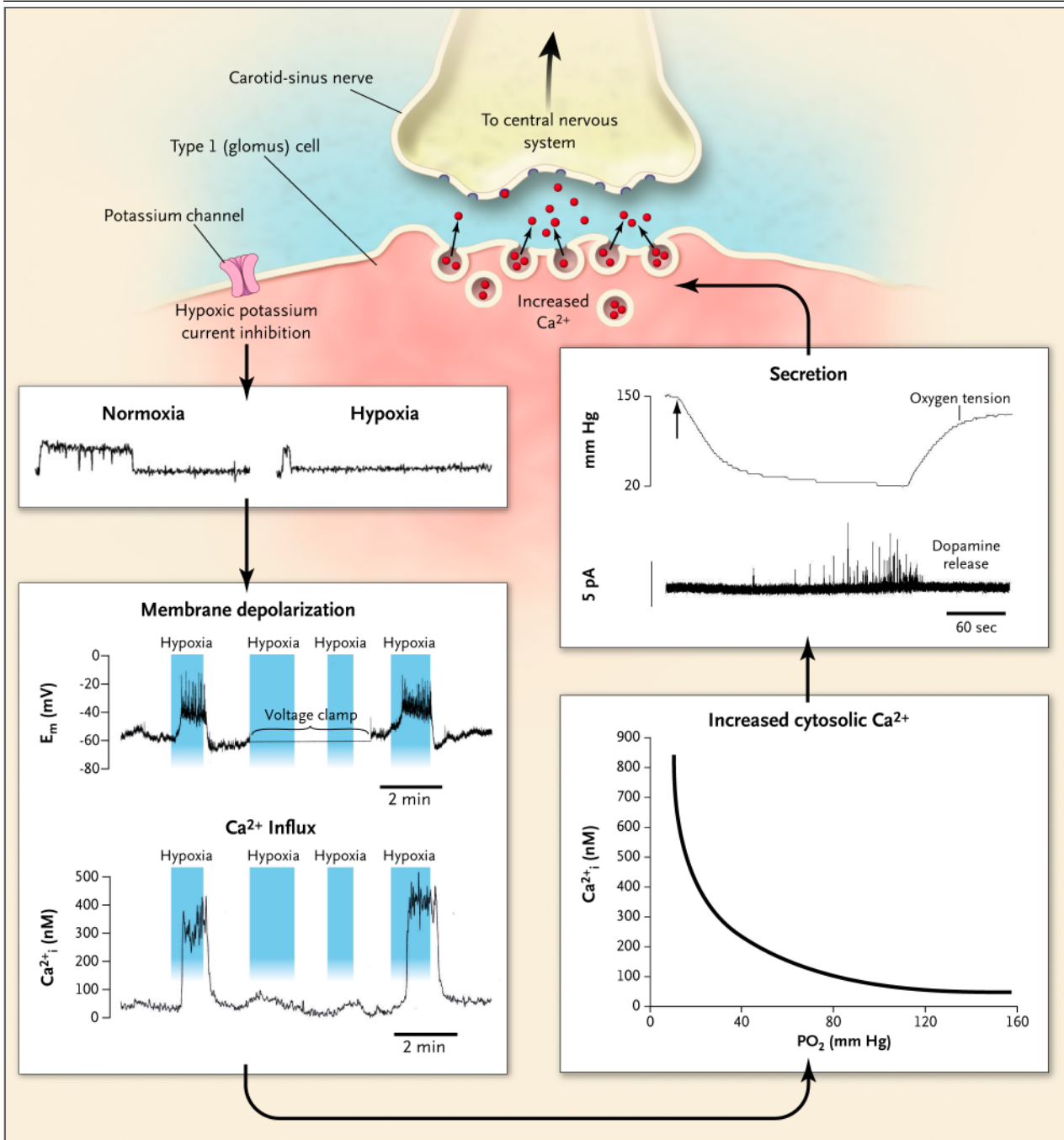


Figure 3. Oxygen Sensing in the Carotid Body.

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 (Ca²⁺), 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.⁶⁴

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.⁶⁵ 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).^{39,66} 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.^{11,67}

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 for⁶⁸ and against^{69,70} 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^{59,71}; 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.⁷² These studies may implicate NADPH oxidase in oxygen sensing. However, mitochondrial depletion prevents hypoxic contraction of single smooth-muscle cells in pulmonary arteries,⁷³ and mitochondrial inhibitors have opposite effects on potassium current and tone in the pulmonary artery and in the ductus arteriosus,^{39,74-76} 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.⁷⁶

MITOCHONDRIA AND OXYGEN SENSING IN THE CAROTID BODY

Inhibitors of mitochondrial function, such as rotenone, stimulate the carotid body,⁷⁷ 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.^{78,79} Moreover, background potassium channels, such as TASK, are sensitive to intracellular ATP, which is depleted by hypoxia.⁷⁹ Although these observations indicate that the carotid body is sensitive to changes in energy metabolism, it is uncertain whether these changes mediate oxygen sensing.⁸⁰ A novel hypothesis suggests that potassium current is maintained during normoxia by carbon monoxide generated by hemoxygenase-2.⁸¹ 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 α subunits of potassium channels or could act through associated β subunits. For instance, cotransfection of Kv β 1.3 with Kv1.5 into green monkey kidney cells (COS cells) inactivates Kv1.5 by facilitating NADPH binding to β subunits; this is reversed by the oxidized pyridine nucleotide NADP.⁸² Hypoxia increases the ratio of reduced to oxidized redox pairs — NADPH–NADP and NADH–NAD — in the lung,^{83,84} which would then inactivate the oxygen-sensitive potassium channels. A similar redox mechanism controls calcium release from the sarcoplasmic reticulum.^{85,86} 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^{87,88} (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

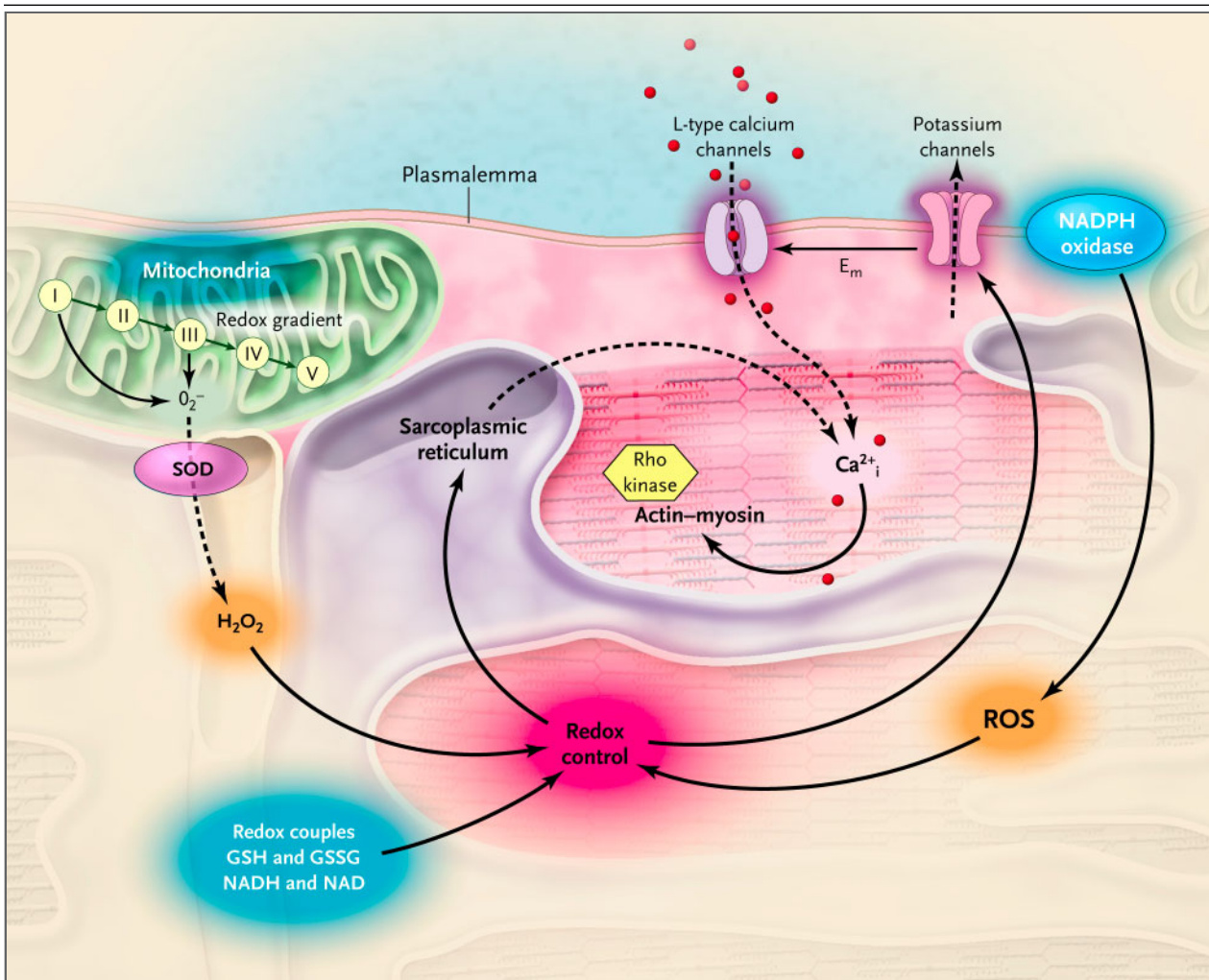


Figure 4. Redox Mechanism for Oxygen Sensing in Specialized Tissues.

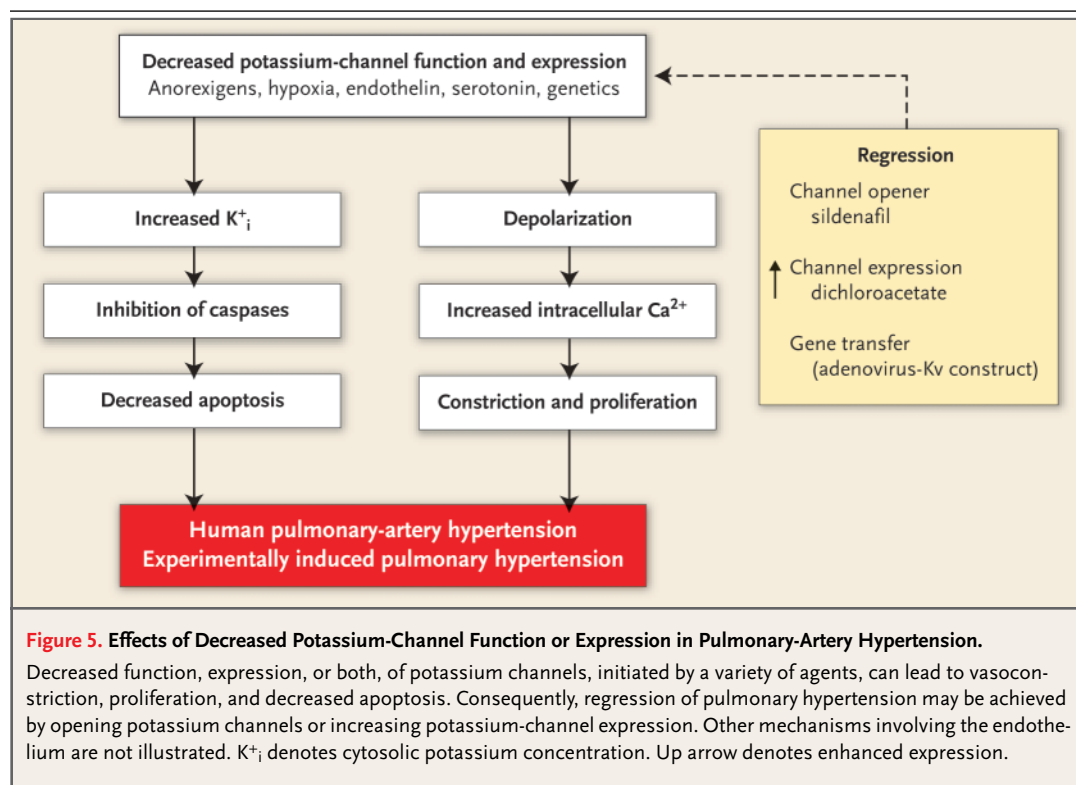
Reactive oxygen species (ROS) from the mitochondria, NADPH oxidase, NADH oxidase, or redox couples may control potassium-channel gating and membrane potential (E_m) and thus calcium entry. The same redox signaling may control calcium release from the sarcoplasmic reticulum. The calcium stores in the sarcoplasmic reticulum, in turn, are replenished by calcium entry through the store-operated channels. Rho kinase augments the response of actin–myosin at any level of cytosolic calcium (Ca^{2+}_i). SOD denotes superoxide dismutase, H_2O_2 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.⁸⁹ This is related in part to abnormal bone morphogenetic protein-receptor signaling, which occurs in some patients with idiopathic pulmonary arterial hypertension.⁹⁰ 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.⁹¹ Conversely, enhancing outward potassium current can initiate apoptosis, and this may be exploited therapeutically to cause regression of pulmonary hypertension¹⁷ (Fig. 5).

The anorectic agent dexfenfluramine has been shown to increase the incidence of idiopathic pulmonary arterial hypertension.⁹² 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.^{93,94} Increased cytoplasmic calcium is an important signal for smooth-muscle-cell proliferation in the pulmonary arteries¹⁷ (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,⁹⁵ dichloroacetate,⁹⁶ and dehydroepiandrosterone,⁹⁷ 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 therapy¹⁹ (Fig. 5).

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



itors by inhalation prevents the deleterious effects of systemic vasodilatation,¹⁰² 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.¹⁰³ 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.¹⁰⁴ Drugs that decrease calcium entry into pulmonary vascular smooth-muscle cells, such as nifedipine, may benefit these patients,¹⁰⁵ and sildenafil, which increases potassium current in smooth-muscle cells of the pulmonary arteries, improves exercise capacity at high altitude.¹⁰⁶

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.¹⁰⁷ 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.¹⁰⁸ 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.¹⁰⁹ Carotid chemoreceptor denervation, which can occur with surgery of the neck, abolishes eupneic ventilatory responses to hypoxia and reduc-

es ventilatory responses to hypercapnia.¹¹⁰ Sudden infant death syndrome could be due in part to changes in the carotid-body chemoreceptors.¹¹¹ Dopamine content in the carotid body is higher in affected children than in normal children,¹¹² and this higher level could cause carotid-body hyposensitivity, since dopamine is known to inhibit calcium current in glomus cells.¹¹³ 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.^{114,115}

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.

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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.

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