Hypoxia. 4. Hypoxia and ion channel function

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Shimoda LA, Polak J. Hypoxia. 4. Hypoxia and ion channel function. Am J Physiol Cell Physiol 300: C951–C967, 2011. First published December 22, 2010; doi:10.1152/ajpcell.00512.2010.—The ability to sense and respond to oxygen deprivation is required for survival; thus, understanding the mechanisms by which changes in oxygen are linked to cell viability and function is of great importance. Ion channels play a critical role in regulating cell function in a wide variety of biological processes, including neuronal transmission, control of ventilation, cardiac contractility, and control of vasomotor tone. Since the 1988 discovery of oxygen-sensitive potassium channels in chemoreceptors, the effect of hypoxia on an assortment of ion channels has been studied in an array of cell types. In this review, we describe the effects of both acute and sustained hypoxia (continuous and intermittent) on mammalian ion channels in various tissues, the mode of action, and their contribution to diverse cellular processes.

Sensing and appropriately responding to changes in O2 concentration is of paramount importance for the survival of all organisms. Over time, O2 homeostasis has evolved into a complex system to regulate O2 delivery and use. While the rapid response to acute reductions in O2 concentration typically results from processes that alter preexisting proteins, such as phosphorylation, stabilization, dimerization, or degradation, durable adaptation to prolonged hypoxic insult requires a coordinated response at the level of gene transcription. Since a deficit in O2 is an important component of various physiological processes and the pathogenesis of numerous diseases, understanding the mechanisms by which changes in O2 are linked to cell function is of great interest.

Ion channels play a critical role in regulating a wide variety of biological processes, including neuronal transmission; control of ventilation; contraction of cardiac, skeletal, and smooth muscle; transport of nutrients and ions across the epithelium; T-cell activation; and glucose metabolism and pancreatic β-cell insulin release. To date, over 300 types of ion channels, differing in their mode of activation (voltage-dependent or -independent, ligand-gated, mechanosensitive, pH) and their ionic permeability (K\(^+\), Ca\(^{2+}\), Cl\(^-\), Na\(^+\)), have been identified. This heterogeneity in ion channel populations provides an astonishing array of variable responses within and between cell types. In 1988, a report demonstrating that rabbit carotid body glomus cells express an O2-sensitive potassium channel ushered in a new era of research exploring whether ion channel activity could be regulated in response to changes in O2 availability (131). Since that initial description of an O2-regulated ion channel, an abundance of work has been produced indicating that a variety of ion channels spread across the different ion channel families are O2 sensitive and exhibit changes in channel activity with acute hypoxia and alterations in channel quantity with prolonged hypoxic challenge. Although a great amount of work has been devoted to the study of hypoxia and ion channels in reptiles, amphibians, and fish, in this review, we will describe the effects of both acute and sustained hypoxia (continuous and intermittent) on mammalian ion channels in several tissues, the mode of action, and their contribution to various cellular processes. It should be noted that in many instances, controversies exist regarding whether the effects of hypoxia on ion channel function are direct or indirect and the exact mechanisms involved in these responses.

Throughout this review, we have attempted to point out possible reasons for conflicting results, including factors such as duration of hypoxic exposure, cell type, experimental conditions, and developmental aspects, all of which can influence the magnitude of responses and may underlie differences in the responses that have been reported.

Continuous Hypoxia

There are a number of physiological and pathological conditions during which tissues are exposed to continuous hypoxia. This can happen for brief periods of time, such as during ischemia, or hypoxia can be for long duration, such as with chronic disease or residence at high altitude. In either case, cells are programmed to rapidly adjust to lowered O2 levels to maintain O2 delivery to critical organs such as the brain and heart. This is achieved, in large part, due to alterations in the activity and, when the hypoxic stimulus is sufficiently prolonged, expression of O2-sensitive ion channels (Table 1), which control a variety of cellular functions including secretion, contraction, excitability, and ultimately, survival.

Chemoreceptors

Acute hypoxia. Breathing rate and compensatory cardiorespiratory adjustments to hypoxia are controlled, to a large extent, by chemoreceptors, which signal the respiratory center to increase breathing frequency and/or lung volume during inhalation. Chemoreceptors are classified as central, located in the medulla oblongata, or peripheral, localized to the carotid bodies and, in some animals, aortic bodies. Although the peripheral chemoreceptors exhibit sensitivity to pH and CO2 (60, 106, 121), the predominant response to changes in CO2 appear to be mediated by central chemoreceptors, since ventilatory responses to CO2 were still present in animals with denervation of peripheral chemoreceptors (88). In contrast, peripheral chemoreceptor denervation eliminates the ventilatory response to hypoxia, indicating a primary role for these chemoreceptors in hypoxia-stimulated breathing responses. Along with pulmonary neuroepithelial bodies in the lung, the
Table 1  Summary of effects of sustained hypoxia on ion channels

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Effect</th>
<th>Subtype</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>Potassium Ion</td>
<td></td>
<td></td>
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<tr>
<td>Kv’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemoreceptors</td>
<td>↓</td>
<td>92, 131, 134, 147, 253</td>
<td></td>
</tr>
<tr>
<td>Neurons</td>
<td>↓</td>
<td>Kv2.1</td>
<td>104, 146</td>
</tr>
<tr>
<td>Heart</td>
<td>↑</td>
<td>Kv1.2</td>
<td>98</td>
</tr>
<tr>
<td>Pulmonary Vasculature</td>
<td>↓</td>
<td>6, 9, 94, 167, 176, 268</td>
<td></td>
</tr>
</tbody>
</table>

| Kv’         |            |         |              |
| Chemoreceptors | ↓ | 169, 185, 252 |
| Neurons     | ↑          | 56, 202, 259 |
| Systemic Vasculature | ↑ | 10, 71, 72 |

| KCa’        |            |         |              |
| Chemoreceptors | ↓ | 223     |
| Neurons     | ↑          | 183     |
| Systemic Vasculature | ↑ | 44, 46, 64, 91, 108, 113, 192, 215 |

| Kir’        |            |         |              |
| Heart       | ↑          | Kir2.1 | 188          |

| TASK        |            |         |              |
| Chemoreceptors | ↓ | TASK-1 | 28          |
| Pulmonary vasculature | ↑ | TASK-1 | 157, 159 |

| Cheremoreceptors | ↓ | 163     |

| VCa’        |            |         |              |
| Voltage gated Neurons | ↑ | 16, 184 |
| Heart       | ↑          | 84, 112, 242 |

| Ca2’        |            |         |              |
| VGCC’       |            |         |              |
| Chemoreceptors | ↑ | L-type | 29, 213     |
| Neurons     | ↑          | L-type | 35, 137, 218, 261 |
| Heart       | ↑          | L-type | 58, 98, 99, 149 |
| Systemic Vasculature | ↓ | 62, 63, 204, 225, 250 |
| Pulmonary Vasculature | ↑ | L-type | 42, 61, 130, 144, 225, 237, 246 |

| Nonselective Cation Channels |            |         |              |
| SOCC’        |            |         |              |
| Chemoreceptors | ↑ | 29     |
| Neurons     | ↑          | 35, 206 |
| Pulmonary vasculature | ↑ | TRPC1,4,6 | 154, 237, 238 |
| Na+ permeable | ↑ | 194, 206 |

See text for definitions and more details.

The identity of the specific K+ channel subtype(s) responsible for chemoreceptor O2 sensing and the mechanism by which this occurs remains in some debate and may vary with species. For example, initial reports in the rabbit carotid body indicated that the O2-sensitive channels were voltage-dependent K+ (Kv) channels (131); however, the fact that inhibitors of Kv channels did not cause depolarization or increase [Ca2+]i, in rat glomus cells suggested that these channels were not active at the resting Em (28, 257) and thus, could not be responsible for initiating depolarization in response to hypoxia. Later reports demonstrated that other K+ channel subtypes, including Ca2+-dependent K+ (KCa), human ether-a-go-go-related gene (hERG), a subtype of inwardly rectifying K+ channels, and twin pore acid-stimulated K+ (TASK) channel-like background currents can be modulated by O2 (28, 162, 169, 170, 252), and since application of inhibitors of TASK-1 (257) and hERG (162) channels caused significant depolarization of the resting Em and elevation of [Ca2+]i, these channels appeared to be excellent candidates for mediating hypoxia-induced depolarization. Consistent with this possibility, inhibitors of hERG and TASK-1 mimic the effects of hypoxia on glomus cells from rabbit (162) and rat (257), respectively, and genetic deletion of TASK-1, but not TASK-3, markedly atten-

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uates the ventilatory response to hypoxia in mice (222). One explanation tying these various reports together is that initiation of depolarization by hypoxia occurs due to inhibition of K⁺ channels open at the resting $E_m$ (i.e., hERG or TASK channels), and that the inhibitory effects of hypoxia on Kv and KCa channels, which normally open with a shift to more positive $E_m$ and/or elevations in $[Ca^{2+}]_i$, serves to limit their activity and maintain depolarization.

Interestingly, these various K⁺ channels have not always been shown to exhibit O₂-dependent regulation, depending on the experimental conditions and the cell type used for heterologous expression (166), signifying complex regulation by hypoxia. Indeed, the mechanism by which K⁺ channels sense changes in O₂ is unclear. In some studies, channels were found to retain their hypoxia responsiveness in excised membrane patches or recombinant expression systems, suggesting the possibility of direct regulation of the channel by O₂ or that the O₂ sensor is closely associated with the pore-forming α-subunits or regulatory β-subunits (110, 160, 166, 167, 185). In this case, such effects could include alteration of cysteine thiols, methionine, or oxidoreductase domains in channel proteins, where changing between oxi- and deoxi-conformations could impact channel gating due to direct allosteric interactions (133, 166). However, findings that the O₂ sensitivity of some channel subtypes depended on the cell type and/or experimental conditions indicates that O₂ sensitivity is not absolutely intrinsic to the ion channels themselves but may be modulated by the interaction between the O₂-sensing signaling molecules and pore-forming subunits.

One possible signaling mechanism linking hypoxia to ion channel function that has received much attention is alterations in reactive oxygen species (ROS). O₂-derived small molecules, including the O₂ radicals superoxide and hydroxyl, ozone (O₃), singlet O₂, and hydrogen peroxide (H₂O₂). ROS avidly interact with a variety of molecules and play an important role in reversible regulatory processes. ROS are produced from a cascade of reactions that starts with the production of superoxide, generated by mitochondrial respiration, xanthine oxidase, uncoupled nitric oxide (NO) synthase, or via reduced nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase (NOX). Superoxide is highly reactive and rapidly dismutates to H₂O₂, either spontaneously or catalyzed by superoxide dismutase. Superoxide can also react with NO to form peroxynitrite, or the iron-catalyzed Fenton reaction leading to the generation of hydroxyl radicals. Of the mechanisms known to produce ROS, evidence suggests that during hypoxia, in many cell types the mitochondria and NOX appear to be the predominate sources.

Supporting a role for the proposal that ROS mediate the effects of hypoxia on carotid body function were studies reporting that application of exogenous oxidants modify K⁺ currents ($I_K$) (3) and several ion channels contain residues that are susceptible to redox modification (6, 43). Since NOX is a major contributor to ROS production in a variety of cells and O₂ supply determines the amount of H₂O₂ formed by NOX, it was proposed that hypoxia decreases H₂O₂ formation, leading to decreased open probability of K⁺ channels, depolarization, and transmitter release (3). Consistent with this hypothesis, application of exogenous H₂O₂ depressed chemoreceptor activity and diphenyleneiodonium, a purported inhibitor of NOX, augmented the basal sensory activity and blocked further augmentation by hypoxia (43). Similarly, ROS generation in glomus cells was reduced by the putative NOX inhibitor 4-(2-aminoethyl)-benzenesulfonyl fluoride (51, 87). However, neither of these inhibitors is specific for NOX, as diphenyleneiodonium can inhibit other oxidases and 4-(2-aminoethyl)-benzenesulfonyl fluoride is a general serine protease inhibitor. Investigators have also used transgenic approaches in an attempt to elucidate whether alterations in NOX-derived ROS production might contribute to K⁺ channel regulation during hypoxia. In mice deficient for the gp91 catalytic subunit of the NOX2 isoform, O₂ sensitivity of airway chemoreceptor cells was impaired (67), but loss of gp91 had no effect on the hypoxia responsiveness of carotid bodies (187) and did not alter glomus cell morphology or the O₂ sensitivity of $I_K$ (86). However, there is evidence suggesting that NOX4, not NOX2, is the primary isoform in chemoreceptors (75). Expression of TASK-1 channels in human embryonic kidney (HEK) cells revealed not only that these currents were inhibited by hypoxia but also that NOX4 colocalized with TASK-1, and that deploration of endogenous NOX4 abolished the O₂ sensitivity of the current (122). Whether this also occurs in chemoreceptors, or whether other NOX isoforms might be involved, is unknown.

Whereas modulation of K⁺ channels by ROS is an attractive hypothesis, several lines of evidence suggest that this may not occur in glomus cells. For example, some investigators found that neither oxidants nor reducing agents impacted carotid body function (4, 76), and O₂-sensitive KCa channels of rat glomus cells respond to PtdOH changes independently of redox modification (185). Also at odds with the hypothesis that a reduction in ROS inhibits K⁺ channels is the finding that acute hypoxia caused an increase ROS production in glomus cells (51, 87). Interestingly, whereas the hypoxia-induced increase in ROS was absent in cells from mice deficient for p47phox (87), these cells exhibited augmented hypoxia-induced alterations in $[Ca^{2+}]_i$ and K⁺ channel activity, results that apparently rule out a functional role for increased ROS in the depolarization and elevation in $[Ca^{2+}]_i$ (87).

The role of mitochondria has also been investigated. In many cell types, hypoxia alters the production of ROS from mitochondria, with labs reporting both decreased and increased mitochondrial ROS generation during hypoxia (for review, see Ref. 34). While it was reported that hypoxia caused mitochondrial depolarization in glomus cells (25) and the hypoxia responsiveness of intact glomus cells was reduced by rotenone, an inhibitor of complex I (158), other inhibitors of the mitochondrial electron transfer chain had no effect, suggesting that the action of rotenone may have been independent of its effects on the mitochondria. Moreover, the hypoxia-induced reduction of $I_K$ was maintained in airway chemoreceptor cells devoid of mitochondria or after mitochondrial inhibition (191).

Thus, whereas there is no doubt that hypoxia exerts an inhibitory effect on chemoreceptor K⁺ channels, the differences in reported results suggest that the exact mechanisms underlying this response remain to be completely defined and that there may be a combination of factors that contribute to hypoxia-induced inhibition of K⁺ channels in chemoreceptors.

**Chronic hypoxia.** With chronic hypoxia (CH), carotid bodies exhibit marked hypertrophy due at least in part to glomus cell hyperplasia. CH also reduces $I_K$ amplitude (85, 90, 253) but increases the density of Na⁺ and Ca²⁺ channels in carotid body glomus cells (89, 211). Recently, detailed molecular
biological and electrophysiological studies have shown that T-type (transient) VGCCs are upregulated by CH in the rat pheochromocytoma cell line (PC12), O₂-responsive cells that release neurotransmitters and possibly in other tissues (48). Interestingly, although the inhibitory effect of hypoxia on whole cell Iᵥ was intact after CH, a specific deficiency of Kᵥ channel activity was noted, leading to loss of depolarization in response to acute hypoxia (253), suggesting that some, but not all, of the O₂-sensitive machinery is impaired by CH.

Central Nervous System

The brain is exquisitely sensitive to hypoxia; induction of hypoxia or anoxia in glial cells and most neurons leads to cell death. Ischemic stroke, where tissue hypoxia is frequently a factor, is often accompanied by neuronal hyperexcitability, which further aggravates brain damage. A number of reports have detailed the effects of ischemia, low glucose, and hypoxia/anoxia on the brain (for review, see Ref. 35). In this review, we will focus only on literature in which the effects of hypoxia and/or anoxia were determined. In nerve cells, most investigators describe an initial hyperpolarization followed by severe depolarization and influx of calcium. The initial, transient hyperpolarization observed in response to hypoxia in hippocampal and dorsal vagal neurons is due to the opening of ATP-sensitive K (KᵥATP) channels (223). KᵥATP channels are active at normal cellular ATP levels, but as ATP is depleted during hypoxia, increased activity of these channels leads to K efflux and hyperpolarization, perhaps in an effort to protect the cells and minimize hypoxia-induced damage by reducing neuronal input (68, 101, 259). A few studies suggest that Kᵥ channels, perhaps activated by release of Ca²⁺ from internal stores, may also participate in the initial hyperpolarization (56, 202, 259). However, sustained hypoxia/anoxia leads to depolarization in hippocampal (184) and hypoglossal (82) neurons. The mechanisms underlying this depolarization are likely to be complex and appear to involve a combination of factors including inhibition of Kᵥ channels and Na⁺ influx via nonselective cation channels (NSCC) or voltage-gated Na⁺ channels. For example, Kᵥ channels are potent suppressors of neuronal excitability; in particular the Kᵥ channel family member Kᵥ2.1 plays a pivotal role in the homeostasis, excitability, and survival of neurons, including hippocampal and cortical pyramidal neurons (20, 52, 141, 151, 164). Brief in vivo exposure to anoxia induces rapid, reversible dephosphorylation of Kᵥ2.1 in brain samples from the cortex and hippocampus due to overactivation of NMDA receptors by excess glutamate (104, 146). A caveat of these experiments is that hypoxia was induced by inhalation of 100% CO₂, which could cofound the results; however, in cultured hippocampal neurons, chemical hypoxia, induced by a mixture of sodium azide and 2-deoxy-d-glucose, but not elevated CO₂, reproduced Kᵥ2.1 dephosphorylation (146). In these experiments, the dephosphorylation of Kᵥ2.1 was mediated by the activation of calcineurin secondary to intracellular Ca²⁺ release (146).

In addition to Kᵥ channels, other studies have shown that a large component of the hypoxia-induced depolarization was due to influx of Na⁺ (150, 184). Hypoxia/anoxia increased intracellular Na⁺ concentration ([Na⁺]ᵢ) in a variety of nerve cells, including those from the cortex (17, 65), hippocampus (184), substantia nigra (80), and hypoglossal cells (109). This increase is due in part to reduced activity of the Na⁺-K⁺-ATPase, likely as a result of lowered ATP levels (184). Voltage-gated Na⁺ channels are also present in neurons, and blockade of these channels attenuated depolarization and cell injury in response to anoxia/hypoxia (16, 184), suggesting that hypoxia activates these channels. Na⁺ influx may also occur through NSCCs, since Na⁺ influx induced by anoxia can be attenuated by blockade of NSCCs (194).

In vitro experiments almost universally demonstrate an elevation in [Ca²⁺], during hypoxia (49, 137, 218, 259, 261), although several factors, including duration of hypoxia, cell type, experimental conditions, and age of the animals influence the magnitude of the response. In neurons, influx of calcium occurs primarily through VGCCs and Ca²⁺-permeable NSCCs, which include store-operated and receptor-operated channels (35). There is substantial evidence that hypoxia-induced activation of VGCCs contributes to the increase in [Ca²⁺], with most data suggesting that high-voltage (L- or N-type) rather than low-voltage-activated (T-type) VGCCs are responsible for Ca²⁺ influx (137, 218, 261). With respect to high-voltage VGCCs, both L-type (long-lasting) and N-type (neuronal) VGCC contribute to the increase [Ca²⁺], during hypoxia/anoxia, with L-type channels exhibiting more sensitivity to hypoxia (137). While depolarization could certainly contribute to increased VGCC activity, the activation of VGCC and resultant increase in [Ca²⁺], observed in hippocampal neurons during application of anoxic perfusate to rat brain slices was found to occur secondary to production of NO (218). Moreover, when L-type currents were examined in cultured hippocampal neurons, NO could directly modulate both the open probability and voltage-sensitivity of the channels (218), which the authors attributed to S-nitrosylation.

It is widely held that the increase in [Ca²⁺], results in cell death; however, in dissociated, CA1 neurons cell injury and death in response to anoxia was not prevented by removal of extracellular Ca²⁺ and rather appeared to be dependent on Na⁺ influx (66, 254). Consistent with these studies, inhibiting voltage-gated Na⁺ channels attenuated depolarization and cell injury in response to anoxia/hypoxia (16, 184). In either case, it is clear that hypoxia has dramatic effects on the central nervous system that are mediated, to a large extent, by the activity of ion channels. Although most of these changes in ion channel function appear to be related to depletion of ATP, it appears that at least some of these effects could be attributable to O₂-sensitive channels.

Similar to the effects reported in neurons, sustained hypoxia/anoxia also leads to depolarization (15) and increased [Ca²⁺], (206) in glia. Little is known about the direct effects of acute hypoxia on K+ channels in glial cells, although in cultured astrocytes, acute hypoxic challenge leads to a ROS-dependent increase in Kᵥ channel activity (260), rather than the decrease in activity that would be consistent with depolarization. Whether depolarization results from direct, active inhibition of other K+ channels or is due to passive inhibition of K+ channels due to increased extracellular K+, is unclear. The increase in [Ca²⁺], in response to hypoxia appears to involve both influx of Ca²⁺ and release of Ca²⁺ from intracellular stores, with Ca²⁺ release initiating Ca²⁺-activated Ca²⁺ entry through NSCCs (206). Since many NSCCs are also Na⁺ permeable, it is possible that activation of NSCC by hypoxia could also contribute to depolarization.
Heart. The mammalian heart is an aerobic organ with one of the highest O₂ consumption rates of any organ (8–15 ml O₂·min⁻¹·100 g tissue⁻¹ at rest), requiring a constant supply of O₂ to maintain essential cellular processes and sustain cardiac function and viability. Hypoxia can alter the cardiac action potential and lead to arrhythmia; thus, the myocardium has developed several defenses that allow these cells to rapidly adjust to changes in O₂. Ion flux is critical to normal cardiac function, and there is significant evidence that hypoxia alters the function of ion channels and membrane transporters in cardiomyocytes.

Resting $E_m$ in the heart is controlled primarily by K⁺ channels, in particular the current carried by inward rectifier K⁺ channels ($I_{KIR}$). Action potentials are generated by depolarization reaching a threshold at which point voltage-gated Na⁺ channels open. Entry of Na⁺ through these channels generates a large inward current ($I_Na$) and rapid depolarization (Fig. 1), resulting in phase 0 of the cardiac action potential. The opening of Na⁺ channels is accompanied by the closing of $I_{KIR}$, which shifts $E_m$ toward the equilibrium potential of Na⁺ ions. When the $E_m$ is sufficiently depolarized, L-type Ca²⁺ channels are activated, allowing Ca²⁺ entry. Repolarization occurs with activation of slow and fast transient outward K⁺ currents ($I_{K_s}$) and inactivation of Na⁺ channels. The late phase of the cardiac action potential results from activation of the rapid ($I_{Kr}$) and slow ($I_{K_s}$) components of the delayed rectifier K⁺ channel as well as $I_{KIR}$, resulting in inactivation of the L-type Ca²⁺ channels. Disturbances in ion channel conductance result in alterations in the shape and duration of the action potential that can lead to cardiac arrhythmias and sudden death.

Hypoxia has been shown to impact the activity of all of the channels involved in cardiac action potential generation. Acute hypoxia increases [Na⁺], due to increased influx through late or persistent $I_Na$ even though fast or transient $I_Na$ are reduced in rat ventricular myocytes (84, 112, 242). With respect to maintaining Ca²⁺ homeostasis, L-type VGCCs provide the main entry pathway for Ca²⁺ into cardiomyocytes and are a major component of excitation-contraction coupling. Hypoxia/anoxia causes a rapid decrease in the basal current carried by L-type Ca²⁺ channels ($I_{Ca}$) (58, 98, 149). Interestingly, hypoxia reversibly reduces peak $I_{Ca}$ without shifting the current-voltage relationship (99). In the rabbit heart, hypoxia-induced early AP shortening and speeding up of the late phase of repolarization are sensitive to low concentrations of Ba²⁺ (188), a specific blocker of $I_{KIR}$, with Kir2.1 channels as the major isoform underlying $I_{KIR}$ in cardiac myocytes. The slow component of the delayed rectifier K⁺ channel ($I_{Ks}$) was also decreased during hypoxia, without affecting the rapid component ($I_{Kr}$) (98). All of these changes could contribute to generation of arrhythmias or alterations in action potential duration.

Most evidence suggests that the effects of acute hypoxia on myocyte ion channels are due to a change in the phosphorylation state of the channel or in the redox status of the cell. Evidence for a direct effect on the channels includes reports that the reduction in VGCC activity occurs both in native cardiomyocytes and in HEK cells expressing a recombinant protein and requires a subset of residues in the cytoplasmic tail (58, 149), where phosphorylation of the channel by protein kinase A (PKA) augments the current and prevents inhibition by hypoxia (149). In addition to direct effects on the channels themselves, hypoxia may also alter the regulation of these channels via indirect mechanisms, such as changes in ROS. ROS levels change during hypoxia, although whether they increase or decrease in cardiomyocytes is still a matter of some debate and may vary due to a variety of factors, including age, localization within the cell, and source of ROS being measured. For example, hypoxia decreased ROS production from NADPH oxidase in adult cells (100, 241), resulting in repression of $I_{Ca}$ (100). This is consistent with reports indicating that ROS can target T-type calcium channels on the sarcolemma and suppress $I_{Ca}$ (81). In contrast, chick cardiomyocytes respond to hypoxia with an increase in ROS (54, 119), which could serve to increase [Ca²⁺], by depressing the activity of the sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA), SERCA2, a membrane calcium pump that has been shown to play a crucial role in cardiac Ca²⁺ handling and as a determinant of myocardial contractility (114). Increased generation of ROS can also alter the activity of cardiac Na⁺ channels, K⁺ channels, and ion exchangers, such as the Na⁺/Ca²⁺ exchanger (NCX) (74, 152).

With respect to prolonged or long-term exposure to hypoxia, changes in channel expression have been noted. $K_{ATP}$ subunit expression in human myocardium is inversely correlated with venous PO₂ and culturing ventricular myocytes under hypoxic conditions resulted in hypoxia-inducible factor 1 (HIF-1)-dependent upregulation of $K_{ATP}$ channel expression (183).

Vasculature. Most blood vessels respond to hypoxia, although the manner of response is dramatically different. With rare exceptions, systemic vessels dilate in an attempt to increase O₂ delivery to tissues, while pulmonary vessels constrict to divert blood from poorly perfused areas to enhance ventilation-perfusion matching. Both mechanisms have been well-studied, with alterations in ion channel activity and/or expression playing a fundamental role in these responses.

SYSTEMIC VASCULARITY. In the systemic circulation, vasodilation in response to hypoxia is largely due to a reduction in

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![Fig. 1. Schematic representing the currents involved in the cardiac action potential. Baseline membrane potential ($E_m$) is controlled primarily by current through inward rectifying K⁺ channels ($I_{K IR}$). Opening of Na⁺ channels ($I_{Na}$) leads to rapid depolarization. Inactivation of Na⁺ channels along with the opening of K⁺ channels leads to a small downward deflection mediated by a transient outward current ($I_o$). The plateau of the action potential is maintained by the balance of an inward Ca²⁺ current through L-type channels ($I_{Ca,L}$) and outward K⁺ current through slow delayed-rectifier K⁺ channels ($I_{K_s}$). As Ca²⁺ channels close, the opening of rapid delayed-rectifier K⁺ ($I_{Kr}$) and K⁺ channels contributes to rapid repolarization. As $E_m$ reaches the resting $E_m$, $I_{K_s}$ and $I_{Kr}$ are inhibited.](http://ajpcell.physiology.org/)
[Ca\(^{2+}\)], in the smooth muscle cells (SMCs), although the response may be modulated by changes in endothelial cell-derived relaxing and contracting factors, the production of which may also be Ca\(^{2+}\) dependent. Numerous studies have indicated that ion channels in the systemic vasculature, both Ca\(^{2+}\) permeable and Ca\(^{2+}\) impermeable, are regulated by hypoxia. Electrophysiological studies in vascular SMCs from systemic arteries demonstrated that hypoxia can exert a direct effect on VGCCs, with a reduction of PO\(_2\) rapidly and reversibly inhibiting \(I_{Ca}\) (63, 204, 225). The inhibition of L-type VGCCs has a direct functional effect as hypoxia-induced relaxation was absent in aortic rings that were pretreated with antagonists of L-type VGCCs (91). In isolated human coronary SMCs, low O\(_2\) tension decreased L-type calcium current and reduced \([Ca^{2+}]_i\) (204). A role for decreased Ca\(^{2+}\) influx through VGCCs during hypoxia has also been demonstrated in smooth muscle from porcine coronary, rabbit cerebral, celiac, and femoral arteries and hamster cheek pouch arterioles (62, 63, 204, 250). The reductions in \([Ca^{2+}]_i\) and relaxation observed in porcine coronary arteries, which were not dependent on changes in K\(^{-}\) channel activity or intracellular pH (168, 195), and the hypoxia-induced changes in \([Ca^{2+}]_i\), observed in individual cells were not associated with depolarization (250), suggesting the possibility that hypoxia directly inhibited \(I_{Ca}\). This was tested in several types of systemic smooth muscle, where hypoxia reversely inhibited VGCC currents (62), even in voltage-clamped cells where changes in \(E_m\) would not be a contributing factor (63), although in hamster cremaster arterial smooth muscle cells, hypoxia had no effect on L-type currents (40). While the reason underlying the difference in response in this study is not clear, the majority of data suggests that hypoxia can exert a direct influence on VGCCs.

In addition to direct modulation of Ca\(^{2+}\) channels, changes in \(E_m\), which is controlled primarily by K\(^{-}\) channels in vascular smooth muscle, can also influence the activity of Ca\(^{2+}\) entry pathways and \([Ca^{2+}]_i\). Consistently, hypoxia induced hyperpolarization of cerebral or coronary arterial muscle cells that results from the activation of \(K_Ca\) and/or \(K_{ATP}\) channels and accounts for the associated cerebral vasodilation (10, 44, 46, 71, 192). Hyperpolarization would serve to decrease Ca\(^{2+}\) influx by reducing the likelihood of VGCC activation. Toward this end, hypoxia has been shown to enhance the activity of \(K_Ca\) channels in cerebral arterial SMCs (71, 72). In an early study, the effect of hypoxia was observed in excised patches and was not associated with a change in \([Ca^{2+}]_i\), in cerebral vascular SMCs from cat (71), suggesting that hypoxia had a direct effect on the channel. However, this was not confirmed in a later study in rat cerebral vascular SMCs (72), indicating that enhancement of \(K_Ca\) was not due to a direct effect of hypoxia on the channel but was mediated by a second messenger. It is unlikely that the \(K_Ca\) channels were activated by an increase in \([Ca^{2+}]_i\), since hypoxia has routinely been demonstrated to decrease Ca\(^{2+}\) levels in cerebral arterial (226) and other systemic smooth muscle. Since the effect of hypoxia on \(K_Ca\) channel activity was blocked by antioxidants, and hypoxia increased superoxide formation in these cells (72), it would appear that redox modulation of \(K_Ca\) channel activity plays a role. In contrast, a hypoxia-induced inhibition of \(K_Ca\) channel currents in cerebral arterial muscle has been reported (72). Studies have suggested that \(K_Ca\) channels located in proximity to sarcoplasmic reticulum (SR) Ca\(^{2+}\) stores are activated by Ca\(^{2+}\) sparks to promote hyperpolarization and relaxation of vascular SMCs (249). Where a reduction in \(K_Ca\) activity in response to hypoxia was reported, it was suggested to be related to reduced sensitivity and/or coupling of \(K_Ca\) channel to Ca\(^{2+}\) sparks, possibly in an attempt to limit hypoxic cerebrovascular dilation (272). Whether these effects of hypoxia on K\(^{+}\) channels truly has a physiological impact remains uncertain, as some studies have found that inhibitors of \(K_Ca\) channels blocked hypoxia induced relaxation (71), whereas other studies have demonstrated that inhibition of these channels had no effect (195).

The loss of ATP or elevation of adenosine by hypoxia has been implicated in eliciting arterial dilation through opening \(K_{ATP}\) channels (113). In coronary artery resistance arteries, hyperpolarization to hypoxia was blocked by \(K_{ATP}\) channel inhibitors (70). In some studies, hypoxia activated gibbenclamide-sensitive K\(^{+}\) currents in amphotericin B-perforated cells of isolated porcine coronary SMCs (44) and hypoxia-induced relaxation was at least partially inhibited by \(K_{ATP}\) blockers in thoracic aorta (91) and cremaster (108), carotid (215), and cerebral (64) arteries. However, in other studies, hypoxia did not increase \(K_{ATP}\) current of voltage-clamped, amphotericin B-perforated cells (108, 182) and hypoxic relaxations were resistant to glibenclamide (204). Whether these variations in findings are due to species and/or vascular bed differences is unclear.

Whereas numerous studies have explored the effect of hypoxia on ion channel function in systemic smooth muscle, only a few studies have focused on endothelial cells. Hypoxia increases \([Ca^{2+}]_i\) in aortic (11, 145) and human umbilical vein (5) endothelial cells, primarily through increased Ca\(^{2+}\) influx. Hypoxia has also been shown to increase \(I_K\), via a mechanism involving Ca\(^{2+}\)/calmodulin-dependent protein kinase (CaMK), presumably secondary to the increase in \([Ca^{2+}]_i\) (13). In this case, hyperpolarization would increase the driving force for Ca\(^{2+}\) entry, perhaps helping to maintain Ca\(^{2+}\) influx. An increase in \([Ca^{2+}]_i\) could lead to increased production of vasodilators, such as NO and prostacyclin, which could participate in hypoxic vasorelaxation.

**PULMONARY VASCULARITY.** A large amount of attention has focused on understanding the role of ion channels in the pulmonary responses to both acute and chronic hypoxia. Whereas acute hypoxic pulmonary vasoconstriction (HPV) is believed to be a compensatory response aimed at matching ventilation with perfusion by shunting blood flow away from poorly oxygenated areas of the lung, prolonged global alveolar hypoxia can occur with residence at high altitude and with a variety of chronic lung diseases and results in an elevation in pulmonary arterial pressure that can eventually lead to right heart failure and death.

**Acute hypoxia.** Since the first detailed reports of HPV emerged over half a century ago (231), much attention has focused on the mechanism. The use of isolated perfused lung preparations, where conditions are such that changes in pressure reflect alterations in pulmonary vascular resistance, demonstrated that decreasing O\(_2\) tension causes an immediate elevation of pulmonary arterial pressure (for review, please see Ref. 244). In more reduced preparations, such as isolated vessels or pulmonary arterial smooth muscle cells (PASMCs), an abundance of research has revealed that while endothelial cells can produce vasodilator and vasoconstrictor substances
that modulate HPV, the sensor and effector mechanisms reside in the PASMCs (for review, please see Refs. 1 and 2). There is also considerable heterogeneity in the magnitude of the hypoxic contractile response between species and in pulmonary vascular smooth muscle from different locations within the lung, with smooth muscle from small diameter, distal pulmonary arteries exhibiting greatest contraction. Thus differences in the species and portion of the vascular tree from which the arteries and/or cells were derived could contribute significantly to variations in reported results.

Although hypoxia-induced increases in Ca\(^{2+}\) sensitivity of the contractile apparatus have been described, most investigators agree that an increase in [Ca\(^{2+}\)]\(_i\) in PASMCs is a key component underlying the contractile response to hypoxia (1, 132, 245, 248), although how hypoxia triggers this response is a matter of some debate. Numerous studies report that HPV and the increase in [Ca\(^{2+}\)]\(_i\) is due to Ca\(^{2+}\) influx, as both are inhibited by the absence of extracellular Ca\(^{2+}\) or blockade of Ca\(^{2+}\) channels (42, 130, 144, 154, 226, 237, 246). In PASMCs from small diameter arteries, VGCCs were found to be activated by lowering of P0\(_2\) (61, 225); however, it is unlikely that the majority of the Ca\(^{2+}\) influx is due to a direct effect of hypoxia on VGCCs. Rather evidence indicates that influx via VGCCs is secondary to depolarization. In PASMCs from adult animals, E\(_m\) is primarily regulated by Kv channels, and several labs have demonstrated that hypoxia inhibits I\(_{Kv}\) (6, 9, 167, 179, 268), with some investigators demonstrating that this was due to Ca\(^{2+}\) release from the SR (179, 180), whereas others suggested that this was due to redox modulation of the channel (6, 247), interactions with regulatory proteins, such as Kv\(_{\beta}\) subunits (174) or phosphorylation by PKC-\(\zeta\) (39).

Numerous attempts have been made to identify the exact Kv family member that is O\(_2\) sensitive. Some studies suggested a prominent role for the Kv1.5 subunit (8, 73); however, this was countered by data from non-PASMC expression systems indicating that expression of either Kv1.5 or Kv1.2 resulted in currents that were insensitive to hypoxia (160, 176) or only inhibited at very high E\(_m\) (103). Interestingly, a hypoxia-sensitive I\(_{K}\) did develop when Kv1.2 and Kv1.5 were coexpressed (103, 160) or when Kv1.5 was expressed in PASMCs (176). These findings suggest that O\(_2\) sensitivity may be determined by the host cell (41, 133). The effects of hypoxia on Kv currents were also inhibited by antibodies to Kv2.1 (9, 94), which produces a current that was inhibited by hypoxia but only in a few cells in non-PASMC expression systems. In PASMCs, Kv2.1 and Kv9.3 coimmunoprecipitate (167) and while Kv9.3 does not form functional channel as a homotrimer, coexpression of Kv2.1 and Kv9.3 produced a current that was inhibited by hypoxia (103, 167). Taken together, the most logical explanation for these findings is that the O\(_2\) sensitive Kv channel in PASMCs is composed of heterotrimers of two or more Kv channel subunits.

In at least one study, the effect of hypoxia on I\(_{Ca}\) was observed in the presence of Kv channel inhibitors, suggesting that activation occurs by mechanisms not involving Kv channels (61); however, the exact Kv channel inhibitor used in this study was not described and leaves open the possibility that only a subset of Kv channels were inhibited. Nonetheless, other K\(^+\) channels have also been implicated in HPV and could contribute to depolarization. Since K\(_{Ca}\) channels are not active at the resting E\(_m\) in PASMCs from adult animals (7, 159, 199, 267), it is unlikely that hypoxia-induced inhibition of K\(_{Ca}\) channels contributes to depolarization. In contrast, a nonactivating, background K\(^+\) current has been characterized in PASMCs that is active at the resting E\(_m\) and is inhibited by hypoxia (57, 157, 159). It is believed that these channels include TASK-1 subunits, since overexpression of these proteins results in a current with similar features (69, 157). Moreover, depletion of TASK-1 by small interfering RNA reduced this current and eliminated its sensitivity to hypoxia (157). The mechanism by which hypoxia inhibits TASK channels remains unclear.

Interestingly, measurements of [Ca\(^{2+}\)]\(_i\) in PASMCs isolated from distal pulmonary arteries revealed that the hypoxia-induced increase in [Ca\(^{2+}\)]\(_i\) was only partially inhibited by VGCC inhibitors but was completely abolished by antagonists of NSCCs (237). Some NSCCs are highly Ca\(^{2+}\) permeable; both store-operated and receptor-operated Ca\(^{2+}\) channels, which are likely composed of canonical transient receptor potential (TRPC) proteins, fall into this category and are expressed in PASMCs (135, 136, 236). Acute hypoxia increased Ca\(^{2+}\) influx through store-operated Ca\(^{2+}\) channels, which may be due to Ca\(^{2+}\) release from the SR (107) or phosphorylation of the channels by Rho kinase or myosin light chain kinase (238). In addition to being Ca\(^{2+}\) permeable, these channels can also conduct Na\(^+\) influx, allowing for the possibility that opening of these channels also contributes to depolarization and activation of VGCC. Since inhibitors of either VGCC or NSCC equally reduce HPV in isolated lungs (246), it is clear that both of these Ca\(^{2+}\) influx pathways contribute to the contractile response.

**Chronic hypoxia.** Prolonged exposure to hypoxia causes depolarization of the resting E\(_m\) in PASMCs (59, 97, 196, 198, 205) and reduces the activity and expression of Kv channels (59, 97, 177, 196, 198, 235, 240) through a mechanism involving both activation of the transcription factor, HIF-1, and the endothelium-derived factor endothelin-1 (196, 251). The discovery that E\(_m\) was depolarized in PASMCs following exposure to CH prompted the assertion that L-type channels would be activated and [Ca\(^{2+}\)]\(_i\) would be increased in these cells, leading to the enhanced PASMC contraction and proliferation characteristic of pulmonary hypertension. Indeed, CH elevates [Ca\(^{2+}\)]\(_i\) (127, 177, 197, 207, 239, 251), but while this can be reduced by L-type channel antagonists early on (251), VGCC blockers have no effect on [Ca\(^{2+}\)]\(_i\), or tone in pulmonary arterial smooth muscle from chronically hypoxic animals (127, 197, 239), indicating that VGCCs no longer play an important role. Instead, inhibitors of NSCCs reduced both [Ca\(^{2+}\)]\(_i\) and tone in pulmonary vascular smooth muscle from chronically hypoxic rats (127, 239). NSCCs are not activated by depolarization, but their activity may be influenced by phosphorylation (93, 156, 238). Increased abundance of TRPC protein was observed in pulmonary arterial smooth muscle from chronically hypoxic animals (127, 239), and in PASMCs exposed to prolonged hypoxia in vitro, through a HIF-1-dependent mechanism (239), leading to a sustained elevation in [Ca\(^{2+}\)]. Thus, while CH certainly serves to inhibit the activity and expression of Kv channels, the role of depolarization is unclear. Instead, reducing K\(^+\) efflux may serve to increase intracellular [K\(^+\)], which has been associated with reduced apoptosis.

**Intermittent hypoxia.** Transient declines in tissue O\(_2\) supply, or intermittent hypoxia (IH), are frequently occurring events in
human life with widespread implications on cell metabolism and the pathogenesis of various diseases. IH most often occurs during sleep, where repetitive occlusions of the upper airways is a hallmark of sleep apnea syndrome with estimated prevalence in the range of 5–15% of the general adult population (181, 263) and as much as 30–40% in obese subjects (227). In the case of sleep-disordered breathing, brief periods of airways collapse leads to rapid, repetitive O₂ desaturations, with the most severe patients experiencing ≥30 hypoxic episodes per hour. A majority of premature infants also suffer from repeated apneas during sleep due to immaturity of central and peripheral breathing regulation (178, 212). Several laboratories have developed animal models designed to mimic the IH experienced during sleep apnea. These models typically expose animals to short durations (several seconds) of moderate hypoxia at a rate of 5–60 cycles/h, 8–12h/day for several days to weeks.

Whereas IH due to repetitive cessation of breathing is perhaps the most well-described pathological condition, periods of IH can also occur in organs affected by atherosclerotic narrowing of arteries and might contribute to hypoxic preconditioning, which has been clearly conceptualized in heart (18, 19, 31) and suggested to occur in the brain (27, 193). In contrast to the short, rapid decreases on oxygenation that occur over long periods of time with sleep apnea, the IH of hypoxic preconditioning typically involves slower cycles of IH (on the order of several minutes to hours). Although both sustained hypoxia and IH are defined by limitation of O₂ supplies, IH exposure is differentiated by limited duration and repeated occurrence in time, and thus, might trigger different cellular adaptive responses, which may also be tissue or cell-type specific (Table 2).

Table 2 Summary of effects of intermittent hypoxia on ion channels

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Effect of Hypoxia</th>
<th>Subtype</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potassium Ion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kᵥca</td>
<td>Neurons</td>
<td>↓</td>
<td>219, 220</td>
</tr>
<tr>
<td>KᵥATP</td>
<td>Neurons</td>
<td>↓</td>
<td>Kir6.1, 6.2</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>↓</td>
<td>14, 223, 258</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑</td>
<td>12, 274</td>
</tr>
<tr>
<td><strong>Sodium Ion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltage dependent</td>
<td>Heart</td>
<td>↑</td>
<td>153, 255, 274, 275</td>
</tr>
<tr>
<td></td>
<td>Neurons</td>
<td>↑ Short</td>
<td>78, 79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Long</td>
<td>117, 233</td>
</tr>
<tr>
<td></td>
<td>PC12</td>
<td>↑</td>
<td>117, 233</td>
</tr>
<tr>
<td><strong>Voltage-Gated Ca²⁺ Channels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCGG</td>
<td>Heart</td>
<td>↑</td>
<td>L-type</td>
</tr>
<tr>
<td></td>
<td>PC12</td>
<td>↑</td>
<td>L-type</td>
</tr>
<tr>
<td></td>
<td>Systemic Vasculature</td>
<td>↑</td>
<td>L-type</td>
</tr>
<tr>
<td><strong>Nonselective Cation Channels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOCC</td>
<td>Systemic Vasculature</td>
<td>↑</td>
<td>232</td>
</tr>
</tbody>
</table>

See text for definitions and more details.

Chemoreceptors

Reflexes arising from the carotid bodies contribute to control of blood pressure, sympathetic activity, and control of breathing. In animal models, exposure to IH using a regimen reminiscent of sleep apnea augments the hypoxia-induced increase in sensory discharge from carotid bodies due to a mechanism requiring mitochondrial and NADPH-derived ROS and increased HIF-1 activity (171–173). Increased carotid body sensory activity was observed with as little as 16h of IH exposure in neonates (173), and when the duration of IH was prolonged (10 days), the augmented hypoxia sensitivity of the carotid body was associated with elevated blood pressure in adult animals (172), suggesting that alterations in carotid body function during long-term IH could contribute to the cardiovascular comorbidities associated with sleep apnea. Although the sensory activity of the carotid body is modulated by ion channel activity, whether IH exposure leads to alterations in channel function or expression in the carotid body is currently unknown; however, given the effects of ROS on various channels and the known presence of O₂-sensitive channels in carotid body, this is certainly a possibility.

Adrenal chromaffin cells, derived from adrenal medulla, and PC12 cells are commonly used O₂-responsive models for investigation of neurotransmitter release. Exposure of PC12 cells to short-term exposure to IH (15–60 cycles) augments the release of neurotransmitters and neurotrophic factors in response to a subsequent acute hypoxic challenge by activation of voltage-gated Na⁺ channels, presumably leading to depolarization and elevation in [Ca²⁺], through both Ca²⁺ influx from extracellular sources via N-type and L-type VGCCs as well as Ca²⁺ release from intracellular stores regulated by inositol trisphosphate (IP₃) and ryanodine (RyR) receptors (IP₃R and RyR, respectively)(117, 233). This response was not observed (117) or blunted (233) when cells were exposed to preconditioning with sustained hypoxia. Similar results were observed in neonatal animals exposed to 5 days of IH, where hypoxic challenge caused increased catecholamine release from freshly isolated medular chromaffin cells related to upregulation of T-type VGCC mRNA, increased IₛCa, and increased RyR mRNA expression (210). Surprisingly, the IH-induced potentiation of hypoxia-induced catecholamine release persisted into adulthood (209). The effect of longer duration IH exposure on hypoxia-induced neurotransmitter/catecholamine release in these cells, and whether a similar response occurs in the carotid body, is unclear.

Short-term IH exposure (up to 120 cycles) in PC12 cells resulted in the activation of HIF-1 due to accumulation of HIF-1α, the O₂-dependent subunit of the HIF-1 transcriptional complex (266). The induction of HIF-1 was found to occur by a process initiated in response to NOX-dependent generation of ROS, which activated phospholipase C, producing the second messenger molecules IP₃ and diacylglycerol (266). Generation of diacylglycerol results in the activation of PKC, which in turn leads to the phosphorylation and activation of the cellular target, mTOR (mammalian target of rapamycin), stimulating synthesis of HIF-1α (266). Additionally, IP₃ acts on IP₃ receptors to induce the release of intracellular Ca²⁺. The elevation in [Ca²⁺]ᵢ not only contributes to activation of PKC (266), but also activation of CaMK, leading to phosphorylation of a regulatory protein, p300, which promotes HIF-1α transcription.
and limited hypoxia-induced elevations in \[Ca^{2+}\] expression of neuronal NO synthase, lower NO production, described (219), all of which can contribute to decreased KCa channel activity and/or expression in this system has not yet been examined.

Central Nervous System

In the brain, it has been suggested that short periods of \(O_2\) deprivation lead to adaptive mechanisms that protect against subsequent longer ischemic events (27). In contrast, long-term IH can have detrimental effects on neuronal functioning (83, 123, 125). Despite the recognized impact of IH, there is a dearth of information regarding IH-mediated effects on ion channels in the brain and central nervous system, and factors specific for the brain, including different regional susceptibility to hypoxia (83, 140, 148) and development/maturity of the brain tissue of neonates, together with variability in duration and type of hypoxic stimulus needs to be taken into account when interpreting the limited data available.

Neurons located in the hippocampus are a frequently investigated neuronal cell type owing to their higher susceptibility to hypoxia-induced morphological and functional changes and relation to neurocognitive dysfunction during IH (83, 123, 125). For example, in newborn mice, 4 wk of exposure to IH decreased the excitability of hippocampal neurons, as determined by reduced action potential firing, lower voltage-dependent \(I_{Na}\), and prolonged deactivation time constant of Na+ channels (78), although opposite results were observed following shorter (2 wk) exposure (79). In contrast to these functional changes in \(I_{Na}\), IH decreased voltage-gated Na+ channel protein expression following a 2-wk exposure, whereas an increase in protein expression was observed after 4 wk of exposure (273). These observations suggest that the changes in \(I_{Na}\) induced by IH are likely due to mechanisms other than simply increasing channel numbers and point to an important interplay between neuronal maturational processes and IH.

KCa channels are widely expressed in brain tissue and, as noted earlier, are responsible for regulation of cellular excitability and neurotransmitter release. It has been shown recently that pharmacological inactivation of KCa channels leads to learning impairment (143), and deficits in learned behavior have also been described in rodents after 14 days of IH (243). It is perhaps not surprising then, that exposure of adult rat hippocampal neurons to IH for several days decreased the activity of KCa channels (219, 220). In this model, reduced expression of neuronal NO synthase, lower NO production, and limited hypoxia-induced elevations in \([Ca^{2+}]_i\) were also described (219), all of which can contribute to decreased KCa channel activity. Additionally, increased ROS production following prolonged IH exposure (186, 219, 228) represents another possible pathway by which KCa channel function might be reduced (190, 220). These results suggest that early long-term IH exposure could have a detrimental impact on cognitive function.

Examination of neurons located in the nucleus of the solitary tract (NTS), which are involved in chemoreceptor-mediated regulation of breathing, revealed downregulation of KATP channel protein expression and limited hypoxia-induced hyperpolarization following 1 wk of IH exposure (269). These findings were interpreted as a counter-regulatory response that, even though possibly not beneficial for the cell, renders these neurons sensitive to chemoreceptor signaling under hypoxic conditions and enables the appropriate respiratory responses. This hypothesis is supported by the observation that NTS neurons derived from IH-exposed rats and obtaining afferentation from the carotid body showed higher excitatory response following glutamate receptor activation compared with normoxic animals (47). In contrast, when the severity of the hypoxia challenge was greater and the duration of IH exposure slightly longer, other investigators observed diminished postsynaptic currents in NTS neurons following IH, which was mediated by reduced presynaptic neurotransmitter release through inhibition of N-type VGCCs by dopamine (118).

Despite overall limited tolerance of neurons to hypoxia, certain regions of brain (dorsal vaginal nucleus) show higher tolerance to hypoxia (14, 15, 83). In contrast to other neuronal populations, where ischemia rapidly leads to terminal depolarization (preceded only by short transient hyperpolarization), these hypoxia-tolerant cells are distinguished by prolonged activation (several minutes) of KATP channels. Such activation leads to hyperpolarization of the plasma membrane, decreased excitability, and lowered metabolic demands (14, 223, 258).

Direct evidence for IH-induced activation of KATP channels in brain is lacking; however, events leading to neuronal KATP activation, including ROS production, NO-induced protein kinase G (PKG) signaling, mitochondrial KATP activation and activation of CaMK (32, 33, 115, 243) were observed to occur in brain following IH exposure (115, 243).

Whereas the effects of IH on brain KCa channels has not been described, high-throughput analysis of the transcriptomic response to a single hypoxia/reoxygenation event identified KCa channels as hypoxia responsive genes in non-astrocyte, non-neuron brain tissue, suggesting the possibility that these channels may be regulated by IH in at least some regions of the brain (23).

Circulatory System

Heart. Research on the effects of IH in the heart follows two major lines: 1) investigation of adverse cardiac outcomes (coronary artery disease, heart failure, hypertension) associated with IH due to sleep-disordered breathing and 2) elucidating the mechanisms contributing to the protective effects of short, repetitive bouts of hypoxia, a phenomenon known as “hypoxic preconditioning.” Literature describing IH-induced changes in ion channel operation entirely represents the second category.

It is well established that ischemia-reperfusion (I/R)-induced damage to the myocardium can be diminished by preexposure of the heart to ischemic periods, the phenomenon known as “ischemic preconditioning” (45, 116). Although ischemic preconditioning involves reductions in the availability of both \(O_2\) and glucose, several reports suggest that the hypoxia is more important for protection (18, 19, 31). In contrast to the experimental paradigms of IH used to model sleep apnea, IH models of hypoxic preconditioning typically involve longer cycles of hypoxia (on the order of several minutes to several hours). In some cases, the duration of exposure to IH is very short (only...
a few hours), although most studies continued daily hypoxic exposures for 1–6 wk. The preventive effect of IH preconditioning on myocardial damage seems to be dose and time dependent, as short exposure (30 min) is not as efficient as longer exposure (1–4 h) and IH cycles where the O2 tension in the inspired air drops as far as 5% (compared with less severe regimes with a minimum FiO2 of 10%) might actually increase ischemic damage (19, 111). Several mechanisms have been suggested to participate in hypoxic preconditioning, including changes in the metabolic pathways, substrate utilization, mitochondrial metabolism, excitation-contraction coupling regulation, production of ROS, decreased apoptosis of cardiomyocytes, increased myocardial capillary density, and activation of protein kinase C (see Refs. 18, 24, and 165 for review). The subsequent paragraphs summarize the impact of IH exposure on ion channel function in the plasma and mitochondrial membranes and SR.

As described earlier in this review, upon depolarization, Ca2+ entry into cardiomyocytes occurs primarily via L-type VGCCs. The increase in [Ca2+]i, is further facilitated by subsequent Ca2+-induced calcium release from the SR, a process tightly regulated by RyR localized on the SR membrane (175). [Ca2+]i, is returned to basal values mainly by reduced influx due to inactivation of VGCCs, ATP-dependent uptake of Ca2+ back into SR by SERCA, and by efflux of Ca2+ across the plasma membrane via the NCX (128). Alterations in these Ca2+-handling pathways, leading to elevated baseline [Ca2+]i, and intracellular Ca2+ overload, represents one of the main factors involved in mediating I/R myocardial damage and contributes to both decreased contractility and electric instability of cardiomyocytes (203). Several studies have demonstrated that the detrimental increases in [Ca2+]i, myocardial damage, and impaired cardiac contractility induced by I/R in rat cardiomyocytes were prevented by both short- (18) and long-term (50, 234, 255, 262) IH preexposure, with the most common experimental regimen consisting of 4–6 h of moderate hypoxia daily for 2–6 wk.

Given the well-documented role of Ca2+ overload in I/R injury, it might be expected that ischemia would enhance Ca2+ influx via ICa. Instead, ischemia causes a reduction in the transmembrane ICa carried by VGCCs, which, together with prolonged inactivation of the channel, contributes to electrical instability and impairs excitation-contraction coupling (124, 138). The effects of short-term IH on VGCC activity during I/R are unclear; however, long-term IH adaptation, which had no effect on basal ICa, lessened L-type VGCC inactivation and facilitated ICa during ischemic challenge (270), changes that might serve to increase electrical stability, improved myocardial contractation, and diminish cardiac electrical remodeling following an ischemic event (53).

Although Ca2+ influx via VGCC might be diminished during I/R, another mechanism that contributes to Ca2+ overload is impaired Ca2+ uptake into the SR mediated by decreased quantity and activity of SERCA (120, 216, 217). After ischemic injury, SERCA protein expression drops to ~60% of its preischemic values. The decrease in SERCA expression can be ameliorated by 2 wk of adaptation to daily hypoxic exposure (6 h/day) (36). The activity of SERCA is inhibited by the SR membrane protein phospholamban. When phospholamban is phosphorylated on Thy1 by CaMK and on Ser16 by PKA and PKG, its inhibitory effects on SERCA are reduced (230).

Ischemia and reperfusion decrease phospholamban phosphorylation, which contributes to diminished SERCA activity and increased [Ca2+]i (256). Preischemic IH adaptation for several weeks increased phospholamban phosphorylation at both sites and ameliorated SERCA dysfunction, resulting in beneficial effects on cardiac contractility and ICa (36, 256). The loss of IH-induced protection after blockade of PKA or CaMK activity (256) suggests that these kinases are critical for the IH-induced phosphorylation of phospholamban in this model. Consistent with this possibility, a rapid increase in PKA activity following hypoxia/reoxygenation was also documented in other cell types (26, 271).

Similar to SERCA, RyR protein expression (particularly RyR2) was decreased during I/R (36); however, other studies found no change in RyR protein following I/R (262). Nevertheless, IH adaptation reduced this response (36) and enhanced RyR activity (36, 234, 262). The mechanisms responsible for these effects have not been fully elucidated, but ROS-induced oxidation and S-glutathionylation of RyR (38, 55, 102, 210, 214) together with phosphorylation by PKA (262) have been suggested. Although an increase in RyR activity following IH exposure might theoretically be hypothesized to further aggravate ischemia-induced Ca2+ overload by increasing Ca2+ release into the cytosol and thus, be at odds with cardioprotection, preserved RyR activity is connected with higher amplitude ICa, shorter time to peak [Ca2+]i, effective excitation-contraction coupling, and preserved contractility of cardiomyocytes (36, 262).

[Ca2+]i results from the balance between Ca2+ influx, uptake, release, and efflux mechanisms. The main mechanism by which Ca2+ efflux occurs in cardiomyocytes is via NCX. I/R leads to decreased forward activity of NCX, or reversal of its function, with subsequent accumulation of [Ca2+]i, prolongation of action potentials, and risk of arrhythmias (139, 229). Based on measurements of Ca2+ transients in cardiomyocytes, long-term IH preconditioning had no effect on baseline (preischemic) NCX activity or protein quantity; however, IH diminished ischemia-induced prolongation of Ca2+ transients either induced by electric stimulation or by Ca2+ release from the SR (262). Furthermore, it has been shown recently that NCX mRNA is increased following IH exposure in mice hearts (37). NCX activity was not influenced by IH when cells were exposed to PKC inhibitors, whereas PKA and CaMK inhibitors had no effect (262). Besides these preconditioning-related effects, NCX plays crucial role in IH-induced development of left ventricular dilation and systolic dysfunction (37).

In addition to modulating Ca2+-handling pathways, the cardioprotective effects of IH have been independently attributed to mechanisms leading to opening of plasma and/or mitochondrial membrane KATP channels (153, 255, 274, 275). Adult and neonatal rats exhibited diminished frequency of ischemia-induced, KATP channel-dependent arrhythmias, smaller infarct size and better postischemic contractility recovery following IH exposure (12, 153, 161). Furthermore, effects of IH adaptation were abolished by administration of KATP channel blockers (glibenclamide, 5-hydroxydecanoate) while KATP channel openers diazoxide (mitochondrial-selective) and pinacidil (general) mimicked the beneficial effects of IH in control animals (12, 274). Putative mechanisms of cardioprotection mediated by KATP channel opening involve: 1) dissipation of the inner mitochondrial Emt with subsequent inhibition of mitochondrial Ca2+ uptake and increased Ca2+ release,
limiting mitochondrial Ca\(^{2+}\) overload (95); 2) generation of ROS (129, 142, 163); and 3) regulation of mitochondrial volume and respiratory chain activity (126).

The hypoxic preconditioning mediated by short-term IH exposure has also been demonstrated to be dependent on activation of HIF-1, as the effects of preconditioning were lost in mice with heterozygous deficiency for HIF-1α (31) or when HIF-1 activation was prevented (22). PKA and CaMK, which contribute to cardioprotection by IH preconditioning (256), have also been implicated in HIF-1 activation (221). Whether HIF-1 exerts its effects via modulation of ion channel expression and whether HIF-1 is involved in the protection adaption afforded by long-term IH remain to be determined.

**Vascularature.** It has been well established that chronic IH exposure leads to the development of systemic and pulmonary hypertension. The mechanisms underlying these responses are unclear, and whether IH alters the activity or function of vascular ion channels has not been rigorously studied. The involvement of L-type VGCCs in IH-induced outcomes in the systemic circulation is indirectly supported by data demonstrating aggravated hypertension in spontaneously hypertensive rats in response to IH (208). Moreover, indirect and uncomprehensive evidence suggests that exposure to the L-type VGCC blocker, nifedipine (208), not only ameliorates IH-induced pulmonary hypertension but also blunts hypertensive responses in spontaneously hypertensive rats (209).

Prolonged exposure to IH results in the development of pulmonary hypertension that is typically less severe than the pulmonary hypertension that develops in response to sustained hypoxia. IH increased pulmonary expression of NOX, and IH-induced pulmonary hypertension was blunted in animals deficient for the NOX subunit gp91phox (155). Whether pulmonary hypertension in this model results from alterations in pulmonary vascular ion channel activity and/or expression has not been studied; however, as discussed above, HIF-1 regulates the expression of several ion channels known to participate in the development of pulmonary hypertension in response to sustained hypoxia, and significant cross-talk between HIF-1α, ROS and NF-κB activation has been described in pulmonary arterial smooth muscle during IH (21).

**Conclusion**

It is clear that hypoxia is a critical modulator of cell function and that many of the effects of O\(_2\) deprivation can be attributed to changes in ion channel activity and/or expression. Most of the reviewed literature is based on animal models and cell culture experiments, which together with differences in exposure methods (atmospheric pressure vs. hypobaric, duration and frequency, severity), contribute to heterogeneity of observed results as well as limits interpolation to other tissues or even species. Nonetheless, because of their wide ranging effects, ion channels are a frequent target in the search for new drugs. Better understanding of the exact ion channels impacted by hypoxia in each tissue and/or cell type, coupled with the development of more specific, tissue-targeted, less toxic channel inhibitors could have important therapeutic implications for a variety of diseases where sustained or intermittent hypoxia is a complicating factor.

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Because of space restrictions, it was not possible to quote all of the excellent studies that have been published with respect to the research described in this review; our apologies to those whose studies were not cited.

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**DISCLOSURES**

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