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11 Oxygen Sensors of the Peripheral and Central Nervous Systems

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Abstract: Neural systems exposed to diminished oxygen availability have a compromised metabolism that leads to pathophysiological changes or neuronal death, depending on the severity and duration of oxygen deprivation. A distributed network of oxygen sensors responds to protect cells by slowing or ameliorating pathophysiological changes and forestalling neuronal death via short-term or long-term changes involving gene expression and the modification of sensors and effectors. In mammalian systems such protective changes are not sufficient to prevent damage under extreme conditions, unlike some hypoxia- and anoxia-tolerant vertebrates which demonstrate oxygen-dependent, reversible reprogramming to protect vital organs such as the brain and heart.

This chapter examines (1) the nature of the signal for oxygen sensors; (2) the molecules used to sense oxygen; (3) how the primary signal is generated, converted, and used in an oxygen-dependent manner; (4) how effector systems function in different cell types; and (5) how oxygen-sensing pathways are interconnected to more general protective stress responses which confer cross-protection for a number of physiological stressors.

While future therapies may focus on the activation of hypoxia-inducible factor (HIF) and its downstream gene products, selected gene products could be administered to reduce neuronal loss and improve recovery after acute insults due to ischemic events and degenerative diseases of the brain and retina. Activation of neuroprotective pathways by oxygen sensors and other physiological stressors could be used as pretreatment to minimize neurotrauma associated with neurosurgical procedures and as an ancillary treatment during early stages of rehabilitation.

List of Abbreviations: HIF, hypoxia-inducible factor; HREs, hypoxia response elements; Hsps, heat-shock proteins; iNOS, inducible nitric oxide synthase; NE, noradrenaline; ODG, oxygen-dependent genes; ROS, reactive oxygen species; TH, tyrosine hydroxylase

1 Introduction

Diminished oxygen availability is a physiological stressor that compromises metabolism by slowing or halting aerobic ATP production and oxygen-dependent enzymatic reactions. In any neural system this can result in pathophysiological changes or neuronal death, depending on the severity and duration of oxygen deprivation. Oxygen-dependent responses mediated by oxygen sensors via their effectors can elicit neuroprotection in the face of significantly decreased oxygen levels, such that pathophysiological changes are ameliorated and neuronal death forestalled. Disruptions to oxygen availability in humans can range from a severe acute insult occurring as a result of heart attack, stroke, and traumatic brain injury to a chronic mild insult occurring as a result of sleep apnea and early stage cardiovascular disease. The prognosis for short-term survival of these events depends upon how well and how quickly the neural systems can be protected or how fast they can recover. There are clearly several functionally related questions that pertain to oxygen-dependent phenomena. (1) What is the nature of the signal sensed by oxygen sensors? (2) Which molecules and molecular pathways are used to sense oxygen? (3) How is the primary signal generated, converted, and used in an oxygen-dependent manner? (4) How do effector systems function in different cell types, particularly in neural systems? (5) How are oxygen-sensing pathways connected to more general protective stress responses which confer cross-protection for a number of physiological stressors?

For the purpose of this chapter, we define oxygen sensors as molecules and proteins that respond to changes in tissue oxygen levels with a direct change in their structure that results in changes in the tertiary structure and function of existing proteins and the upregulation of oxygen-dependent genes (ODG) often as a result of transcription factor function. Thus, we consider the whole sequence of events from primary oxygen-sensing to oxygen-dependent effects and their significance to equate to oxygen sensors. Consequently, we cover heme-containing proteins (globins and cytochromes), membrane-bound ion channels, mitochondria and their subtypes which appear to be multifunctional (sensing oxygen-dependent signals, generating signals to be sensed by other oxygen sensors and as an oxygen-dependent effector site), cellular systems, and specialized organs such as the carotid body. It is also apparent that oxygen sensors are located in a number of cellular compartments ranging from extracellular sensors (including erythrocytes as sensing

cells) to cytoplasmic compartments (neuroglobin) and even intranuclear domains as exemplified by cytoglobin distribution in the mammalian brain. Also, evidence for interactions between oxygen-dependent and other stress pathways are discussed.

Since molecular oxygen is rarely sensed as a pure signal this review will discuss which additional signals can be sensed when oxygen level approaches a critical threshold, and how changes in the level of some molecules (metabolites) such as reactive oxygen species (ROS) (including hydroxyl radicals) and nitric oxide (NO) as well as changes in NADPH, ATP, pH, and general redox status can modulate the activity of the oxygen sensors and their downstream effectors. We will also examine the extent of cross-talk between oxygen-dependent pathways and pathways originating from other sensors detecting physiological status because it is difficult to separate them entirely; for example, energy-dependent pathways are profoundly altered when oxygen levels fall below a critical threshold.

There are two important points to note throughout. First, while single-cell organisms such as bacteria and yeast contain a single oxygen-sensitive molecular pair to regulate the expression of oxygen-sensitive genes (reviewed by Bunn and Poyton, 1996) no universal oxygen sensor has been identified in vertebrates (reviewed by Wenger, 2000; Lopez-Barneo et al., 2001; Lutz and Prentice, 2002; Cummins and Taylor, 2005; Lahiri et al., 2006). Instead, there appear to be several different primary oxygen sensors which are linked to a number of different effector systems that synergistically mediate oxygen-dependent responses in which neurotransmitters and modulators may or may not be involved.

Secondly most studies on oxygen sensors have used hypoxia as a stressor to elicit oxygen-dependent changes. Usually the physiologically effective degree of hypoxia has not been characterized in detail because the actual oxygen level experienced at the cellular level was not measured. The oxygen tension experienced by any cell in an organism depends on its location, especially its distance from an arterial blood vessel, and on its oxygen consumption. Consequently, there are marked differences of cellular oxygen tensions between cell types even in a normoxic body. For example, at normal ambient oxygen levels, arterial P_{O_2} is approximately 100 mmHg while the P_{O_2} in muscle interstitium *in vivo* has been measured at 3.3–24.2 mmHg and the P_{O_2} at the muscle mitochondria was 4–20 mmHg (Richmond et al., 1997). The mean cerebral P_{O_2} is higher than that of muscle, at around 20 mmHg and the P_{O_2} at the renal medulla is close to 50 mmHg (Johannes et al., 2006). These P_{O_2} values can be considered to reflect normoxia for the respective sites because this is the $[O_2]$ at which the animal displays its routine metabolic rate. Thus, it is clear that normoxia is very different depending on whether the frame of reference is a particular tissue or the mitochondria within that tissue (see also review by Lutz and Prentice, 2002). Furthermore, the oxygen tensions experienced by lung epithelial cells and hepatocytes are markedly different. Since vertebrates characteristically display a heterogeneous oxygen map, perhaps one should define hypoxia as an oxygen tension that is below the tension at which aerobic metabolism becomes limited, i.e., the critical P_{O_2} . In this paradigm, muscle hypoxia would be below 2.4–2.9 mmHg, its critical P_{O_2} (Richmond et al., 1997) and hypoxia for the mitochondria would be below its critical P_{O_2} of 1 mmHg (Rosenthal et al., 1976). Similarly, *in vitro* work on established cell lines should take into account the origin of the cell type used in the definition of hypoxia. In terms of oxygen-dependent responses, it is also important to differentiate between decreased oxygen level (hypoxia) and anoxia, i.e., total lack of oxygen (Wenger and Gassmann, 1996).

In defining hypoxia as the $[O_2]$ at which aerobic metabolism becomes limited, one also needs to consider how limited the aerobic metabolism needs to become, before oxygen sensors initiate signal cascades which result in the upregulation of oxygen-dependent genes and whether general physiological stress *per se* can turn on what are currently thought of as oxygen-dependent genes. We discuss the possibility that whenever anoxic conditions prevail, a response attributed to the involvement of oxygen sensors could be a more general stress response, which has little to do with changes in oxygen tension.

2 The Nature of the Signal Sensed by Oxygen Sensors

Among the systems that are usually classified as oxygen sensitive, only ones that include heme-containing molecule (Lopez-Barneo et al., 2001) or prolyl/asparagine hydroxylase use molecular oxygen as a substrate (Berra et al., 2006). In addition, the level of ROS may be the primary signal that is affecting the activity of

oxygen-dependent systems. While earlier on it was thought that ROS are mainly conferring oxygen toxicity, recent findings indicate that at low levels they are important signaling molecules (Finkel, 1998; Weir et al., 2002; Werner, 2004; Wolin et al., 2005). The effects of ROS are often considered to be mediated via effects on conserved cysteine residues (Michiels et al., 2002). Especially hydrogen peroxide and hydroxyl radicals appear to be important in signaling (Gloire et al., 2006). Hydrogen peroxide may be important, since it is quite stable, membrane permeant (Lesser, 2006), and can affect the activity of tyrosine phosphatases by oxidizing cysteines in the catalytic center (Gloire et al., 2006). Hydroxyl radicals can also carry out the oxidation, and their use in cellular signaling would introduce spatial resolution in the system, because the short life time of the molecule (10^{-7} s) restricts the diffusion distance to 4–5 nm (Lesser, 2006). Notably, it is very difficult to separate hydrogen peroxide and hydroxyl radicals, since hydrogen peroxide is converted to hydroxyl radicals in the Fenton reaction, if adequate iron (or copper) ion stores are available (Bogdanova and Nikinmaa, 2001; Lesser, 2006). While NO has been recognized as an important signaling molecule, less emphasis has been paid to the fact that NO can affect the oxygen affinity of mitochondrial function (Koivisto et al., 1997). Also, NO can react with superoxide anion, and the formed peroxynitrite anion is a powerful membrane-permeant oxidant (Fridovich, 1986; Marla et al., 1997) with a lifetime near 0.1 s. Consequently, NO-dependent pathways may play a role in any effect that is considered oxygen sensitive. Two other gaseous molecules may play a role in oxygen-dependent signaling, namely carbon monoxide (CO) and hydrogen sulfide (H_2S). Heme oxidase enzyme, which has CO as one end product, is regulated by hypoxia (Lee et al., 1997). CO may control the neural discharge from rat carotid body (Lahiri and Acker, 1999). H_2S is involved in the oxygen-dependent regulation in vascular tone (Olson, 2005). Any marked decrease in oxygen availability leads to a decrease in cellular ATP concentration (Lutz and Nilsson, 2004), which appears to be the first step in induction of neural death (Lipton, 1999) and does not occur in the very anoxia-tolerant crucian carp (Lutz et al., 2003) or the tropical epaulette shark (Renshaw and Dyson, 1999). Thus, any mechanism detecting disturbances in the energy balance with oxygen depletion would be highly useful for maintaining cellular function in general and neural function in particular. From an energetic point of view the ADP/ATP ratio is the primary regulated function, but since the AMP/ATP ratio varies as a square of the ADP/ATP ratio, it enables a more sensitive regulation of the energy balance, with the end product that the energy-producing/consuming systems are adjusted (Hardie, 2003). AMP kinase senses changes in AMP and, ultimately, the AMP/ATP ratio, (Hardie, 2003; Hardie et al., 2006), which can thus function both in oxygen-and energy-dependent signaling.

3 Primary Oxygen Sensors

3.1 Heme-Based Molecules

Proteins containing a heme moiety and capable of binding molecular oxygen have been identified in diverse taxa from bacteria to vertebrates. Heme-based proteins sense oxygen by binding it reversibly and thereafter initiating a number of signaling cascades with several second messenger molecules, which can ultimately lead to altered gene expression via the activation of specific transcription factors (Wenger, 2000). The blockade of the oxygen-dependent responses by CO, which binds with very high affinity to the oxygen-binding site of many heme proteins, is taken as confirmation that heme proteins act as oxygen sensors (Zhu and Bunn, 1999). Similarly, if the ferrous iron in the heme group is replaced by cations, such as cobalt, which do not bind oxygen, the effect of such replacement on second messenger systems and transcription mimics that of exposure to hypoxia (reviewed by Wenger, 2000). Also, the suggestion that oxygen sensing is heme based is supported by the observation that treatments of cells with iron chelators such as desferroxamine results in a hypoxic response (Wang and Semenza, 1993; Ho and Bunn, 1996).

Simple heme-containing oxygen sensing molecules that respond to environmental changes are present in microbial symbionts (of plants), regulating genes associated with nitrogen fixation. The rhizobial FixL/FixJ system consists of a protein, histidine kinase, and its response regulator, FixJ, which act as an oxygen-sensitive switch. Oxygen binding to the heme moiety of FixL inactivates its kinase

activity. This prevents the phosphorylation of FixJ and inhibits its downstream signal transduction pathway (Nakamura et al., 2004).

Thermodynamic studies have shown that both ferrous and ferric forms of the oxygen sensor FixL have a significantly lower oxygen affinity than myoglobin (Rodgers and Lukat-Rodgers, 2005). The evolution of high affinity extracellular and intracellular oxygen sensors in higher organisms may have facilitated formation of more complex body plans which include a nervous system and the occupation of a wider variety of niches.

3.2 The Globin Family of Heme Proteins

Circulating oxygen carriers, hemerythrin, hemocyanin, and hemoglobin, are used by many invertebrates to facilitate oxygen transport from environment to tissues. Members of the globin family serve this function in vertebrates. Oxygen causes a direct change in the structure of these proteins and, thus, they can be considered oxygen sensors. The globins bind oxygen to a Fe-containing porphyrin ring. The binding is often cooperative, if the molecule containing globin is composed of subunits. Of the different globin molecules, myoglobin, cytoglobin, and neuroglobin have been detected in the brain and other neural systems, and thus may have different neuroprotective functions.

3.2.1 Myoglobin

Myoglobin is not only found in muscle, but its isoforms are also expressed in other tissues which have a high metabolic rate and correspondingly high oxygen demand such as liver, gill, and brain (Fraser et al., 2006). Interestingly, expression of the unique myoglobin isoform in neural tissue did not change in response to environmental hypoxia (Fraser et al., 2006) unlike the hypoxia-sensitive upregulation of myoglobin in the heart (Roesner et al., 2006). It has been suggested that constitutive levels of myoglobin isoforms may have other functions in nonmuscle tissues. For example, myoglobin may act as a cytoprotective agent to forestall injury during hypoxia/ischemia and reoxygenation/reperfusion by scavenging free radicals (Mammen et al., 2006). Importantly, myoglobin also plays a role in intracellular oxygen diffusion from the cell surface to mitochondria (Wittenberg and Wittenberg, 2003; Roesner et al., 2006).

3.2.2 Cytoglobin

Cytoglobin has been localized to the nucleus in the cells of several tissues (Geuens et al., 2003). The cytoglobin gene contains both hypoxia response elements (HREs) and mRNA stabilization sites characteristic of an oxygen-regulated gene. Furthermore, real-time quantitative PCR has confirmed that cytoglobin is regulated by hypoxia-inducible factor (HIF)-1 α (Fordel et al., 2004).

While cytoglobin is upregulated in response to hypoxia in hippocampal cells *in vitro* (Fordel et al., 2004), it is not uniformly upregulated throughout the brain *in vivo* and it is expressed in different brain regions from those in which neuroglobin is found (Mammen et al., 2006). There is no increased expression of cytoglobin mRNA or protein in the neocortex after either chronic or intermittent hypoxia (Li et al., 2006). The brain regions expressing significantly elevated levels of cytoglobin in response to hypoxia are the areas of the archicortex that are sensitive to hypoxia and oxidative stress, namely the hippocampus, thalamus, and hypothalamus. This provides evidence that cytoglobin is an oxygen-responsive globin *in vivo* (Mammen et al., 2006). Cytoglobin is strongly expressed in the developing mammalian central nervous system (CNS) and it has been suggested that its localization in brain areas sensitive to oxidative stress could be related to a myoglobin-like role in scavenging free radicals associated with the metabolism of oxygen and nitrogen (Mammen et al., 2006).

3.2.3 Neuroglobin

Another recently discovered and characterized intracellular globin, neuroglobin, is expressed in the mammalian central and peripheral nervous systems and has recently been detected in cultured astrocytes from newborn mouse brain (Chen et al., 2005). In ischemic astrocytes, apoptosis increased when cultured cortical astrocytes were treated with neuroglobin antisense (Chen et al., 2005). It has been suggested that neuroglobin is not only associated with areas of high metabolic rate (as indicated by their elevated mitochondrial density) in the brain but also in other neuronal compartments (Hankeln et al., 2005). Neuroglobin mRNA and protein can be detected in neuronal perikarya, axons, and synapses (Hankeln et al., 2005). Neuroglobin makes up less than 0.01% of the protein in the brain (Mammen et al., 2002).

Mammen and coworkers (2002) suggested that neuroglobin expression is correlated with regions of the CNS involved in adaptive stress response pathways. Neuroglobin is found in brain areas that have high levels of nitric oxide (Mammen et al., 2002). Since one of the cytoprotective actions of myoglobin is to detoxify nitric oxide in the heart, it has been suggested that neuroglobin may act similarly in the brain (Mammen et al., 2002; Burmester and Hankeln, 2004). While neuroglobin is strongly expressed in the subthalamic nucleus, only low levels are found in the hypoxia-sensitive cerebellum and hippocampus (Pesce et al., 2004). Data from radiolabeled RNA probes and *in situ* hybridization suggest that neuroglobin expression is related to oxygen-dependent functions. There is a constitutive level of neuroglobin present in brain areas that respond to changes in oxygen levels, for example: the locus coeruleus, the parabrachial complex, and the periaqueductal grey (reviewed by Mammen et al., 2002). The highest levels of neuroglobin have been reported in the mitochondria-rich photoreceptors of the retina (Hankeln et al., 2005).

Neuroglobin has an oxygen affinity characterized by a P_{50} value of 2 torr, which is twofold higher than that of myoglobin but much lower than that of most hemoglobins. Neuroglobin has been implicated in the storage and intracellular transport of oxygen in highly metabolically active tissues (Burmester et al., 2000; Couture et al., 2001; Trent et al., 2001) and in facilitating O_2 diffusion into mitochondria (Burmester et al., 2000). It seems likely that it could function as an intracellular oxygen sensor as well as participate in the metabolism of reactive species such as NO and ROS. Burmester and Hankeln (2004) suggested that neuroglobin could be involved in the destruction of ROS especially in hypoxic conditions or resulting from oxidative stress that follows hypoxia and reperfusion. However, this suggestion appears not to hold for retina, since there was not a significant increase in neuroglobin mRNA and protein in zebrafish (*Danio rerio*) retina following hypoxia, even though the brain neuroglobin level increased fivefold (Roesner et al., 2006). The lack of neuroglobin upregulation in the retina following hypoxia may be species specific since some hypoxia-tolerant animals such as crucian carp can turn off visual processing at low oxygen levels (Johansson et al., 1997). It has also been suggested that neuroglobin could function as a terminal oxidase to regenerate NAD^+ (Milton et al., 2006), and thereby maintain ATP production when oxygen levels are diminished. Neuroglobin has been shown to have neuroprotective properties demonstrated by reduced neuronal damage following stroke (Sun et al., 2003). Furthermore, it is upregulated in the brain of the anoxia- and hypoxia-tolerant turtle, and it has been suggested that it mediates neuronal survival under anoxia (Milton et al., 2006). This protective effect was diminished by the inhibition of neuroglobin expression with an antisense oligodeoxynucleotide, and enhanced by neuroglobin overexpression (Sun et al., 2003). Fago and coworkers (2006) suggest that since neuroglobin reacts with ferric cytochrome *c* with rapid kinetics it may well have a role in preventing apoptosis following periods of neuronal stress that cause a surge in the release of cytochrome *c* from mitochondria.

Neuroglobin induction may also be a part of the stress response initiated by heme-based transcription factors or their second messengers, because a transient increase in neuroglobin level was observed in *in vitro* cultures of cortical neurons after they were exposed to priming conditions that usually result in HIF-1 α upregulation such as severely diminished oxygen levels or the addition of cobalt chloride or the iron chelator, desferrioxamine (Sun et al., 2003). However, neuroglobin may be regulated by response elements other than HREs (see Hankeln et al., 2005 for a review). Neuroglobin mRNA can be induced directly by hemin in a time- and concentration-dependent manner via a nonhypoxia-dependent second signal transduction pathway that can be blocked by the protein kinase G inhibitor KT5823 (Zhu et al., 2002). This suggests that neuroglobin may be a multifunctional protein that is upregulated by hypoxia and

downregulated by protein kinase G. The upregulation of neuroglobin by hemin may represent a HIF-1 α -independent pathway and further research is needed to clarify the interaction of HIF with hemin-induced neuroglobin upregulation. Furthermore, Zhu and coworkers (2002) demonstrated that the hypoxic induction of neuroglobin could be prevented by the mitogen-activated protein kinase inhibitor, PD98059, revealing that neuroglobin expression is regulated by more than one signal transduction pathway. Notably, mitogen-activated protein kinases are involved in redox-dependent signaling.

3.3 Cytochromes

The involvement of cytochromes in oxygen sensing has been indicated in many studies (Duranteau et al., 1998; Ehleben et al., 1998; Porwol et al., 2001; Guzy et al., 2005; Guzy and Schumacker, 2006). The cytochromes involved may be mitochondrial (Guzy et al., 2005, 2006) and nonmitochondrial, e.g., cytochrome aa_3 (Porwol et al., 2001), in origin. In both cases, it appears that ROS are the actual transducing molecules for the cytochrome signal. It also appears that CO may significantly regulate the oxygen-dependent cytochrome function (Porwol et al., 2001).

3.4 NADPH Oxidase

Some of the cytochromes suggested to take part in oxygen sensing are parts of the NADPH oxidase enzyme. NADPH oxidase is a heterodimeric flavocytochrome of g22-phox and gp91-phox, which is capable of recruiting the polypeptides p67-phox, p47-phox, and p40-phox to form a membrane bound, multi-subunit structure (Dahan et al., 2002). Recent findings indicate that there are several isoforms of NADPH oxidase in many cell types and that components of some isoforms can act as putative oxygen sensors (reviewed by Acker, 2005). The signaling is mediated via oxygen-regulated ROS formation. NADPH oxidase inhibitors blocked the response of carotid chemoreceptor discharge to hypoxia (Cross et al., 1990) and blocked K $^+$ and Ca $^{2+}$ currents in type 1 cells of the carotid body (Wyatt et al., 1995). Similarly, mRNA for NADPH oxidase and voltage-gated K $^+$ channels have been colocalized to hypoxia-responsive pulmonary neuroepithelial bodies and it has been suggested that this tight association represents an oxidase-linked K $^+$ channel, which acts as an oxygen sensor (Wang et al., 1996). Furthermore, gene knockout studies have shown that gp91-phox null mice had a significantly impaired hypoxic ventilatory response because of the decreased sensitivity of pulmonary neuroepithelial bodies (Kazemian et al., 2001). However, the gp91-phox knockouts show no impairment of carotid body function (Roy et al., 2000) or pulmonary vasoconstriction (Archer et al., 2000) indicating that this single polypeptide is not pivotal in all effector tissues and it is likely that effectors may contain multiple oxygen sensors. If one sensor malfunctions then compensatory changes can be made by other oxygen responsive sensors. Since NADPH oxidase appears to be involved in oxygen-sensitive responses both in plants and in animals, it may be a widespread evolutionarily conserved oxygen sensor.

3.5 Prolyl and Asparagine Hydroxylases

Transcriptional regulation by oxygen is mainly achieved via the function of HIF. To a large extent, its function is regulated either by affecting the stability of the protein by hydroxylation of conserved prolines (proline 402 and 564 in the human protein) and consecutive degradation of the molecule or by affecting the interaction of the molecule with p300 and consecutive DNA binding as a result of hydroxylation of a conserved asparagine residue (Asp803). However, it now appears that in hypoxia-tolerant animals the transcription of HIF may also play a role in the regulation of the HIF pathway (see Shams et al., 2004; Law et al., 2006; Rissanen et al., 2006b). Both prolyl and asparagine hydroxylases use molecular oxygen as a substrate. Their function is oxygen dependent, and thus they function as oxygen sensors affecting hypoxia-inducible gene expression as first demonstrated by Ivan and coworkers (2001) and Jaakkola and coworkers

(2001). The function of prolyl hydroxylases has recently been reviewed by Fandrey et al. (2006). Three types of oxygen-dependent proline hydroxylases (PHD1–3) have been described; the presence of a fourth (PHD4) has been deduced on the basis of genomic information (Oehme et al., 2002). While hydroxylation of conserved prolines (in places of the protein which have the LXXLAP sequence) is achieved by the prolyl hydroxylases, it appears that several other residues are important for the proper functioning of the PHDs (Fandrey et al., 2006). This indicates that in addition to the properties of the enzymes themselves, the three-dimensional structure of HIF-1 α affects the hydroxylation. Notably, hydrophobicity plots of various vertebrate HIF-1 α s show that the conserved proline residues are in a highly hydrophobic environment, suggesting that the residues are folded within the protein (unpublished results, Rytönen K, Vuori KAM, Primmer CR and Nikinmaa M., 2007). Thus it is possible that the effect of ROS and calcium on HIF-1 α function is mediated by their effect on the three dimensional structure of the protein near the conserved prolines and their consequent accessibility to prolyl hydroxylases. This mechanism would add another way for regulating HIF-1 α function by oxygen, since ROS levels can be oxygen dependent. It would also explain, why HIF-1 function has been shown to be ROS dependent in several studies (reviewed by Haddad, 2002; Kietzmann and Gorlach, 2005; Acker et al., 2006), although the enzymatic hydroxylation and consecutive proteasomal breakdown of the protein do not require ROS (Fandrey et al., 2006).

Oxygen-dependent regulation of the DNA binding of HIF is achieved via the function of asparagine hydroxylase (FIH; factor inhibiting hypoxia-inducible factor) (Kaelin, 2005). Because the prolyl and asparagine hydroxylases have different oxygen affinities, it is possible that different genes are regulated by the two enzymes (Dayan et al., 2006). Also, it is possible that the two enzymes regulate HIF function at different oxygen levels.

3.6 AMP Kinase

The function of AMP-activated protein kinase has been reviewed recently by Hardie (2003). The enzyme is composed of three subunits: the catalytic α subunit and the regulatory β and γ subunits. While the system is activated by AMP, it remains inactive even in the presence of this allosteric effector, if not phosphorylated at a critical threonine residue (Hardie, 2003). Because the enzyme is activated by decreasing energy charge (as occurs in hypoxia and anoxia), it is activated by any form of stress that affects energy production/consumption. Thus, much research on AMPK has been directed toward glucose/glycogen/diabetes and fat metabolism studies (Kim et al., 2005; Yun et al., 2005). AMPK function is also affected by ROS (Choi et al., 2001) and NO (Lei et al., 2005), adding to the potential interactions between different regulatory pathways. Since AMPK is involved in regulating cellular energy balance, its activation switches off energy-consuming and switches on energy-producing pathways. One of the major oxygen-consuming processes in cells involves mRNA translation to proteins. Notably, it is inhibited by AMPK in hypoxia also independently from HIF regulation (Liu et al., 2006), showing the importance of energy sensing in hypoxia regulation.

4 Effector Systems

There are basically two types of effector systems involved in oxygen-dependent phenomena: those that exert their oxygen-dependent effect immediately and those that mediate slower oxygen-dependent changes, e.g., at the transcriptional level. The former are often coupled to ion channels that immediately alter neural functions to exert acute effects, for example, ion channels regulate the responsiveness of respiratory neurons involved in peripheral oxygen sensing associated with ventilatory regulation. On the other hand, slower, chronic effects can regulate neural function in such a way that either anaerobic energy production is facilitated or energy is spared usually via oxygen-dependent gene regulation. Often such gene regulation involves HIF. Notably, oxygen-dependent effects may rely on signals in the form of ROS and energy metabolites in addition to molecular oxygen, so that interactions between oxygen, oxidative stresses, and energy metabolism may occur both in acute and chronic oxygen responses.

4.1 Oxygen-Dependent Ion Transport Systems

4.1.1 Exchangers and Cotransporters

Oxygen-dependent cotransporters and exchangers have been studied in most detail in erythrocytes (Gibson et al., 2000) with only sporadic information on other cell types (Tuominen et al., 2003). In erythrocytes, it appears that a major factor regulating oxygen-dependent ion transport is the hydroxyl radical, which has been suggested to increase the activity of KCl cotransport (Bogdanova et al., 2003) and decrease the activity of Na^+/H^+ exchange (Nikinmaa et al., 2003). While data in mammalian and bird erythrocytes have generally been compatible with hemoglobin being the proximal oxygen sensor (Honess et al., 1996; Muzyamba et al., 1999; Muzyamba et al., 2000; Drew et al., 2004; Flatman, 2005), work on hypoxia-intolerant teleost fish species, such as rainbow trout, indicated that in this species bulk hemoglobin could not be the oxygen sensor (Berenbrink et al., 2000). Interestingly, studies on the erythrocytes of the hypoxia-tolerant crucian carp indicate the presence of two different oxygen sensors, one of which has an oxygen affinity similar to (bulk) hemoglobin, and the other has a much lower affinity (Berenbrink et al., 2006). Thus, the data are compatible with minimally two different oxygen sensors of which one may respond to molecular oxygen as the sensed molecule and the other may respond to oxygen radicals (Bogdanova et al., 2001; Berenbrink et al., 2006).

4.1.2 Ion Channels

Most work on the oxygen-dependent ion channels has been done on excitable cells. Some voltage-gated ion channels, such as K^+ , Ca^{2+} , and Na^+ channels, have conductances that are regulated by oxygen-dependent factors with the result that the excitability of a cell is oxygen regulated. Fast adaptive changes that compensate for diminished O_2 levels are mediated by oxygen-responsive ion channels and are manifested as changes in excitability, secretion or contractility depending on the cell type (Lopez-Barneo et al., 2001). Cysteine-rich voltage gated ion channels respond to changes in reduced/oxidized redox pairs such as NADH/NAD^+ so that they close when oxygen levels drop, favoring the formation of more NAD^+ (Archer, 2000).

A decrease below the threshold P_{O_2} , normally close to 50 Torr, in glomus cells of the carotid body or in the neonatal ductus arteriosus results in an inhibition of the tonic K^+ current. Such oxygen-regulated inhibition of K^+ channels, which may be mediated by mitochondria-derived hydrogen peroxide (Archer et al., 2004), results in an increase in cellular excitability, increased Ca^{2+} influx, and a resultant increase in the level of Ca^{2+} in the cytosol (reviewed by Lopez-Barneo et al., 1999).

Neuroepithelial bodies found in mammalian airways have oxygen-sensitive K^+ currents and are putative airway chemoreceptors that detect changes in the level of O_2 in the airway lumen (Wang et al., 1996). Exposure to acute hypoxia *in vivo* (Lauweryns et al., 1987) or *in vitro* (Fu et al., 2002) results in serotonin release, which may have a role in controlling pulmonary vascular tone. The oxygen sensitivity of these channels may result from their close localization to membrane-bound cytochromes (Youngson et al., 1997). It is clear that the closure of voltage-gated K^+ channels by hypoxic exposure has implications for neuroprotection as demonstrated by K^+ channel arrest in hypoxia-tolerant turtles (Pek and Lutz, 1997; Bickler and Buck, 1998; Hochachka and Lutz, 2001; Bickler and Donohoe, 2002), which reduces Ca^{2+} influx (Bickler and Buck, 1998). In some anoxia-tolerant species, neuronal energy is not only conserved by ion channel arrest but also by ATP-sensitive mitochondrial K^+ channel arrest (reviewed by Buck and Pamenter, 2006). This may have implications for clinical interventions.

In the carotid body, hypoxia causes increased Ca^{2+} influx through voltage-gated K^+ channels. This results in an increased afferent input to the brain stem and consequent neurosecretion of catecholamines. Conversely, in the ductus arteriosus a rise in P_{O_2} triggers increased H_2O_2 production. This inhibits voltage-gated K^+ channels and leads in turn to vasoconstriction and the closure of the ductus arteriosus (Archer et al., 2004). Calcium entry also occurs through L-type Ca^{2+} channels involving PKC and/or phosphatase-sensitive pathways (Summers et al., 2000). It is this Ca^{2+} entry through voltage-gated Ca^{2+} channels that

mediates hypoxia-responsive neurosecretion by the carotid body. The hypoxic response in the carotid body can be blocked by Ca^{2+} channel blockers (reviewed by Lopez-Barneo et al., 2001). Chronic exposure to hypoxia upregulates T-type voltage-gated Ca^{2+} channels as one of the actions of hypoxia-inducible transcription factors (Del Toro et al., 2003).

Neurons in the caudal hypothalamus appear to be oxygen responsive. They alter their firing rates in hypoxia to contribute to the control of cardiorespiratory responses to hypoxia (Horn and Waldrop, 1997). Oxygen-sensitive Na^+ channels appear to regulate neuronal excitability in the caudal hypothalamus, since, as oxygen levels drop below threshold, the Na^+ current is inhibited by a PKC-dependent mechanism (O'Reilly et al., 1997). Prolonged hypoxia can result in Na^+ channel excitability (Xia et al., 2000), depending on the frequency, intensity, and duration of the hypoxic episodes (Zhao et al., 2005). Furthermore, a subset of voltage-gated Na^+ channels, which are tetrodotoxin sensitive, are not inhibited by hypoxia and generate "persistent" sodium currents, which could cause irreversible neuronal damage as Na^+ homeostasis is lost (Hammarstrom and Gage, 2002). Under experimental conditions the "persistent" sodium current can be inhibited by reducing agents such as dithiothreitol and reduced glutathione (Hammarstrom and Gage, 2002) suggesting that the current is, in fact, redox sensitive.

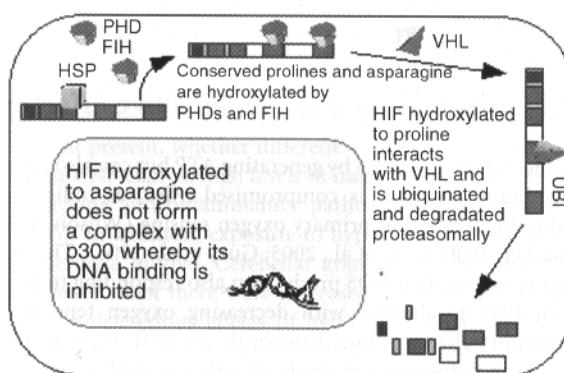
4.2 Hypoxia-Inducible Factor-Dependent Pathway

In the 1990s, oxygen-dependent gene expression was conclusively demonstrated. The early studies concentrated on the erythropoietin pathway (Semenza and Wang, 1992), but later studies have indicated that oxygen directly affects the expression of many, maybe a hundred, genes (Lahiri et al., 2006). The master regulator of oxygen-dependent gene expression is the hypoxia-inducible transcription factor (HIF), which regulates transcription in the following fashion (Wenger, 2000; Bracken et al., 2003) (see  Figure 11-1). The active factor is a nuclear dimer of HIF α and ARNT (HIF β). While both ARNT and HIF α are continually produced at all oxygen tensions, HIF α is rapidly broken down in normoxia. Consequently, the short half-life of HIF α confers hypoxia sensitivity to the function of the protein dimer. As discussed above, HIF α degradation involves prolyl hydroxylases (Ivan et al., 2001; Jaakkola et al., 2001) that bind at conserved proline residues in the oxygen-dependent degradation domain. It appears that the actual core of the oxygen-dependent degradation domain (including proline564 and proline402 (human nomenclature) which undergo oxygen-dependent hydroxylation by prolyl hydroxylases (Semenza, 2001) is invariable in HIF-1 α s across vertebrates (Rytönen K, Vuori K.A.M., Primmer C.R. and Nikinmaa M.; unpublished observations). The regulation of oxygen-dependent degradation may, however, also requires residues in the vicinity of ODD, which affect the tertiary structure of ODD core. Prolyl hydroxylation enables the interaction of HIF α and the von Hippel-Lindau protein, subsequent ubiquitylation, and proteasomal degradation. Recent results suggest that although prolyl hydroxylation does not require ROS, it can be under redox control by hydroxyl radicals (Liu et al., 2004). In hypoxia, prolyl hydroxylation does not occur, whereby HIF α protein is stable and is transported from cytoplasm to nucleus, where it forms a dimer with ARNT and recruits the general transcriptional activator, CBP/p300. Recruitment of transcriptional activators depends on hydroxylation reactions of conserved asparagine (Mahon et al., 2001). Thereafter, HIF (HIF α +ARNT) binds to HREs present in the promoter/enhancer region of the hypoxia-inducible genes, and gene transcription is stimulated. Since both the stability of HIF and its transcriptional activity are affected by oxygen-dependent enzymes, the oxygen affinity of the gene expression depends on the oxygen affinities of both enzymes. Oxygen availability may further affect the three dimensional structure of HIF protein, since both the DNA binding and the transcriptional activation by HIF appear to be under redox control (Lando et al., 2000; Bracken et al., 2003): serine-to-cysteine mutation at a specific residue in the DNA-binding domain confers redox sensitivity of DNA binding, and nuclear redox regulation by Ref-1 potentiates the hypoxic induction of a reporter gene (Lando et al., 2000). At present, while the principles of how HIF regulates gene expression are clear, the fact that oxygen can affect HIF function at several different places and the lack of measurements means that there is currently no decisive information on the possible differences of oxygen affinities for oxygen-dependent gene expression between tissues and species. Also, the

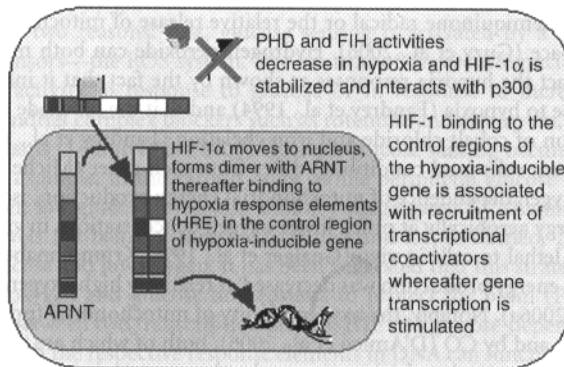
Figure 11-1

A representation of hypoxia-inducible factor (HIF) function. In normoxia, HIF-1 α is broken down after prolyl hydroxylation and its interaction with p300 is diminished after asparagyl hydroxylation. In hypoxia, hydroxylase enzymes are inhibited, whereby the DNA binding of HIF may occur and oxygen-sensitive genes can be induced

Normoxia



Hypoxia



HIF pathway can be stimulated in normoxic conditions by various growth factors, hormones, and cytokines (Richard et al., 2000; Page et al., 2002; Kodama et al., 2003; Ma et al., 2004). In most cases the basis of the stimulation is not known.

4.3 Hypoxia Response Element

In addition to the properties of HIF itself, the HREs especially in the promoter/enhancer regions of the transcribed gene, affect gene expression. HREs may also be present in the introns of oxygen-dependent genes (Rees et al., 2001). The minimal consensus HRE is A/GCGTG (Camenisch et al., 2002). In some cases the presence of HREs alone is not sufficient for hypoxic induction of the genes (Firth et al., 1995), but additional elements such as binding sites for AP1, ATF1/CREB1, HNF4, or Smad3 may be required (Bracken et al., 2003).

4.4 Redox-Responsive Transcription Factors

While HIF has been studied most with regard to hypoxia-inducible gene expression, several other transcription factors also appear to be hypoxia sensitive (Cummins and Taylor, 2005). These include NF- κ B,

which is a redox-sensitive transcription factor (Fan et al., 2003; Fratelli et al., 2005) that consequently responds to variations in ROS, the level of which is dependent on oxygen level (Bogdanova et al., 2003). Observations suggest that while HIF induces gene expression especially in hypoxia, NF- κ B exerts its major influence in hyperoxia (Michiels et al., 2002). Other redox-sensitive transcription factors include PPAR γ , Nrf2, AP-1, STAT, and p53 (Kim and Surh, 2006). Many of these show interaction with HIF (Pan et al., 2004) and can be affected by prostaglandins (Kim and Surh, 2006).

4.5 Mitochondrial Function as an Oxygen-Sensitive Effector System

Mitochondria provide the fuel for life processes by generating ATP but can also provide the signals for death via apoptosis if their membrane potential is compromised. Also, as indicated above, mitochondrial cytochromes are suggested to be involved in primary oxygen sensing (Wilson et al., 1994; Zhu and Bunn, 1999; Chandel and Schumacker, 2000; Guzy et al., 2005; Guzy et al., 2006). The oxygen sensor function of mitochondrial cytochromes results in their ROS production also responding to changes in oxygen. Both an increase and a decrease in ROS production with decreasing oxygen tension are feasible. When the mitochondrial electron transport chain is slowed down or arrested, there is a decreased generation of ROS coupled with an increase in reducing equivalents, so either or both could act as signals generated by mitochondria in response to low oxygen. An increase in ROS production in hypoxia can occur if oxygen affects the lifetime of the ubisemiquinone radical in complex III in the mitochondrial inner membrane and its ability to access the ubisemiquinone radical or the relative release of mitochondrial ROS to the matrix versus intermembrane space (Guzy et al., 2006). Hydrogen peroxide can both mimic hypoxia (Canbolat et al., 1998) and counteract the hypoxia responses as shown by the fact that it inhibited the induction of erythropoietin in response to hypoxia (Fandrey et al., 1994) and that the blockade of Epo expression could be reversed by the addition of cobalt chloride and iron chelation (Fandrey et al., 1997). It is possible that mitochondria from different cells (and conditions) differ from each other (Michelakis et al., 2002).

With regard to the oxygen dependence of mitochondrial energy production, isolated mitochondria are capable of producing energy aerobically at much lower oxygen concentrations *in vitro* than they do *in vivo* resulting in energy levels lethal to cells *in vivo* (Gnaiger et al., 1998; Krumschnabel et al., 2000). In intact cells, the (mitochondrial) energy production was decreased at relatively high oxygen tensions (more than 30 mmHg) (Rissanen et al., 2006a). Notably, the oxygen affinity of mitochondrial function is affected both by NO (Koivisto et al., 1997) and by CO (D'Amico et al., 2006), both of which are molecules associated with oxygen sensing. In addition to mitochondrial energy production, apoptosis (programmed cell death) is also oxygen sensitive and affected by mitochondrial function (Araya et al., 1998; Banasiak et al., 2000). The hypoxic induction of apoptosis often involves release of cytochrome *c* from mitochondria, which initiates consequent caspase activation (Chae et al., 2001). It appears that ROS are involved in the hypoxic apoptosis signaling (Kim and Park, 2003). Also, the dependency of apoptosis on oxygen levels shows interaction with NO, CO, and glucose, indicating interdependence of different cellular effector pathways (Madesh et al., 1999; Malhotra and Brosius, 1999; Tofighi et al., 2006).

4.6 Interactions Between Oxygen-Dependent and Other Effector Pathways

Considering the interconnected nature of biochemical pathways, it is not surprising that antagonistic and synergistic effects of other effector pathways on oxygen sensors have been reported. In many cases, a number of stressors act simultaneously so the integrated response may differ in direction or intensity from that caused by a single stressor, either diminishing the oxygen-sensitive response or enhancing it. Furthermore, the effect of cross-protection by stressors needs to be taken into account when considering physiological responses to a specified stressor because oxygen sensors may be affected by more than one type of stress. For example, physiological stress caused by exercise can increase the generation of both ROS and NO, which are key signaling molecules in the oxygen-dependent pathways as discussed above. It has recently

been demonstrated that exercise at physiologically relevant levels could result in significant elevations in HIF-1 α protein levels coupled with decreased protein levels of the von Hippel–Lindau tumour suppressor, which is responsible for its turnover (Ameln et al., 2005). So it is conceivable that the neurodegenerative and neuroprotective effects of exercise may be mediated by activation of the oxygen-sensing pathway.

Both NO and CO may affect (and be involved in) oxygen-dependent phenomena. Accordingly, *in vitro* experiments have demonstrated that elevated levels of NO or CO can diminish effects of hypoxia on gene transcription. These molecules both activated the internal oxygen-dependent degradation domain and repressed the C-terminal transactivation domain of HIF resulting in less HIF-1 α that can bind to DNA (Huang et al., 1999). On the other hand, *in vivo* experiments have demonstrated that NO is one of the modulators of the hypoxic ventilatory response mediated by the rostral mediolateral medulla (de Paula and Branco, 2003). It is not clear at present, whether different cell types respond in a different manner.

Activated microglia in the CNS express high levels of inducible nitric oxide synthase (iNOS) and occur in high numbers in brain ageing and inflammatory pathologies such as stroke and neurodegenerative diseases (Mander et al., 2005). In addition, exposure to hypoxia and oxidative stress can further increase the number of activated microglia *in vivo*. Cerebellar granule cells in culture were more susceptible to hypoxia-induced neuronal death when there were increased levels of NO present (Mander et al., 2005). Thus, NO may potentiate the deleterious effects of hypoxia by blocking the action of HIF-1 α on the induction of neuroprotective genes via the diminished levels available to bind to DNA, as discussed above (Huang et al., 1999).

The brain, approximately 2% of the body mass, has a high metabolic demand which accounts for approximately 50% of total body glucose utilization. Cross-talk between diminished oxygen and glucose levels occurs for at least two reasons: first, glucose sensing is activated by a signaling pathway that is common with oxygen sensors—the ROS system. The neurons of the arcuate nucleus that modulate insulin release show increased activity in response to glucose or in response to increased ROS as a result of treatment with mitochondrial complex blockers such as rotenone and antimycin, which can be reversed by treatment with antioxidants (Leloup et al., 2006). Second, glucose sensing occurs in some cells which also sense oxygen levels, for example, in the carotid body. While peripheral glucose levels are monitored and responded to by the liver, low glucose levels also inhibit voltage-gated K⁺ channels in the glomus cells, as occurs when oxygen levels fall below a threshold (Pardal and Lopez-Barneo, 2002). Since glomus cells respond to both low glucose and low oxygen, it has been suggested that this strategically placed multifunctional sensor regulates oxygen and glucose homeostasis to protect the brain (Pardal and Lopez-Barneo, 2002). Also, interaction between two transcription factors, HIF and glucose-dependent transcription factor, has been described in which the respective response elements in DNA can function both as glucose response elements and HREs (Kietzmann et al., 2002).

The interaction between oxygen-dependent pathways and more general stress-sensing pathways can be seen at an intracellular level, for example in the upregulation of molecular chaperones such as heat-shock proteins (Hsps), which assist in refolding damaged proteins (Burston and Clarke, 1995; Lund, 1995) and maintaining the tertiary structure of proteins during metabolic stress (Gonzalez et al., 1991). Hsps are upregulated from their constitutive level in response to a diverse array of physiological stressors (Basu et al., 2002). These stressors include exposure to psychoactive drugs (Miller et al., 1991), neurodegenerative disease (Harrison et al., 1993), cellular injury (Liang and MacRae, 1997), hypoxia, ischemia (Welch, 1992; Locke and Noble, 1995), and acute temperature change (Airaksinen et al., 1998). In fact, many studies have shown the accumulation of Hsps in hypoxia/anoxia (Patel et al., 1995; Hammerer-Lercher et al., 2001). In many of the studies that show hypoxic/anoxic accumulation of Hsps, it is difficult to say if the response is a general response to stressful conditions or specific to oxygen limitation. The theory of parsimony predicts that a broad range of triggers probably converge on a single molecular target, which once activated serves to upregulate Hsp production and while this remains to be fully tested, there is evidence that induction of Hsp70 is linked to both an oxygen sensor and/or an energy sensor since Hsp70 promoter activation responds to low oxygen (Madamanchi et al., 2001) as well as to decreased cellular energy charge (Kiang and Tsokos, 1998). Furthermore, energy sensors and oxygen sensors appear to act synergistically to regulate Hsp70 levels and it was suggested that a metabolic sensor may be involved in further upregulating the level of Hsp70 above the level that could be induced by anoxia alone (Renshaw et al., 2004). Similarly,

cross-protection occurs: when animals are exposed to a sublethal physiological stressor, they are protected from a subsequent stressor of the same or a different modality. In the mammalian heart, heat treatment activates HIF-1 α and its target genes including EPO, which may then be responsible for the observed cross-protection to infarction after ischemia reperfusion (Maloyan et al., 2005). In the brain, exposure to brief ischemia made the hypoxia-sensitive CA1 neurons of the hippocampus tolerant to levels of ischemia that were normally lethal (Kitagawa et al., 1990; Kirino, 2002). In addition, nonischemic insults, which resulted in the induction of Hsps in the brain and retina, have been associated with resistance to a variety of insults including ischemia (Franklin et al., 2005). Taken together these data illustrate the multifunctional nature of oxygen-sensitive molecules and suggests that many may be part of the repertoire of responses to stress in general rather than to a specific stressor.

Interaction between oxygen-dependent and other responses occur both in rapid and slower responses. For example, the rapid oxygen-dependent regulation of membrane transport appears quite often to be regulated by ROS, and thereby redox state. Thus, any redox disturbance will affect oxygen-dependent ion transport. As indicated above, gene expression—a slower oxygen-dependent system—is also affected by redox-state dependent transcriptional regulation.

4.7 Oxygen-Dependent and Xenobiotically Induced Gene Expression Pathways

With regard to the function of HIF, its dimerization partner ARNT is also a dimerization partner for many other transcription factors, among them the aryl hydrocarbon receptor (AhR; dioxin receptor). When it was observed that both the xenobiotically induced and hypoxic gene expression used the same dimerization partner, several studies investigated the possibility that one affected the other. While some studies have not been able to show interaction, several others have indicated interaction which may be cell type specific (Gradin et al., 1999; Gassmann et al., 1997; Chan et al., 1999; Nie et al., 2001). There may also be differences in which pathway is preferred (Hofer et al., 2004). In addition to the interaction between aryl hydrocarbon receptor and HIF pathways, studies have indicated an interaction with HIF-pathway and the pathway involved in the generation of diel rhythmicity (Chilov et al., 2001).

4.8 Oxygen- and Temperature-Dependent Responses

Interactions between temperature responses and hypoxia-induced responses may also occur. The possibility of this interaction is of minor importance for the CNS of homeothermic animals, since CNS temperature is usually tightly regulated. If effects in homeotherms are observed, they will be apparent in the peripheral nerves of animals living in cold climates, since there the temperature may be decreased. In contrast, the temperature of all the tissues of poikilothermic animals may show large fluctuations. However, even in mammals temperature affects HIF-1 α expression. Increased amounts of HIF-1 α protein with concomitant induction of Hsp90 and Hsp70 have been observed in mice exposed to heat (Katschinski et al., 2002), upon heat acclimation in rat (Maloyan et al., 2005) and in human hepatoma cell lines exposed to heat (Katschinski et al., 2002). In poikilotherms, increased expression of HIF occurs during the heat acclimation of *Caenorhabditis elegans* (Treinin et al., 2003). Association of HIF- α with the Hsp heterocomplex Hsp90–Hsp70 stabilizes HIF-1 α by protecting it from degradation in both normoxia and hypoxia (Minet et al., 1999; Katschinski et al., 2002; Katschinski et al., 2004). It appears that Hsp90 binds to PAS-B domain of HIF- α and exerts a stabilizing influence on the protein (Katschinski et al., 2004). Whereas the above studies have indicated interaction of the heat-shock response and the HIF response, recent studies (Rissanen et al., 2006b) have indicated that acclimation to a reduced temperature is associated with an increased level of Hsps of the 70 and 90 classes, and increased HIF function, as shown by increased DNA binding of the transcription factor, in a poikilothermic vertebrate, crucian carp. The close association between increased Hsp expression and increased HIF reveal the interaction of temperature and stress-sensitive pathways with oxygen-sensitive pathways.

5 Cellular Systems Described for Oxygen-Dependent Phenomena

5.1 Nonexcitable Cells: Erythrocyte as an Example

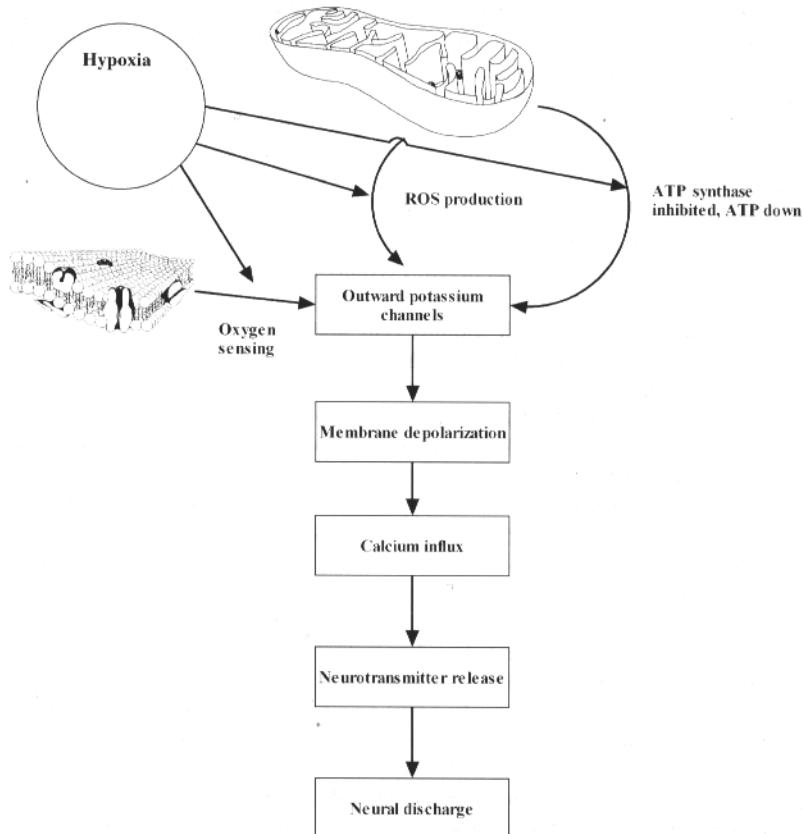
Among nonexcitable cells, erythrocytes have been studied in most detail with regard to oxygen-dependent ion transport, and the function of the ion transport pathways has been reviewed by Gibson et al. (2000) (see also **Sect. 4.1**). As to the physiological role played by the oxygen-dependent pathways, they regulate oxygen transport in hypoxia, and possibly in hyperoxia (Nikinmaa, 2003). While data do not allow firm conclusions to be made about the role that erythrocytes may play in oxygen sensing, the interaction between NO and hemoglobin, and the possibility of consequent blood flow regulation (Stamler et al., 1997), open up the possibility that regulation of erythrocytic oxygen transport is utilized in the regulation of oxygen-dependent responses.

5.2 Excitable Cells: Carotid Body Glomus Cell as a Primary Example

Carotid body glomus cells are cells that sense oxygen in the arterial chemosensory organ. Because of their behavior as oxygen sensors of the peripheral nervous system, they are involved in the control of breathing and have become probably the most important single excitable cell type in which oxygen sensing has been studied. Oxygen sensing by carotid body glomus cells and consecutive mechanisms behind oxygen-dependent nervous signaling to CNS have been the subject of several reviews (Acker and Xue, 1995; Gonzalez et al., 1995a, b; Bisgard, 2000; Prabhakar and Overholt, 2000; Lahiri et al., 2001, 2006; Lopez-Barneo, 2003). Basically, in acute hypoxia, changes in oxygen level are sensed, the activity of potassium channels depends on the oxygen level sensed, leading to an increased efflux of potassium and membrane depolarization in hypoxia. Consequently, calcium influx occurs via calcium channels, neurotransmitters are released, and increased neural discharge occurs in hypoxic conditions (Lahiri et al., 2006). The sequence of oxygen-dependent responses of carotid glomus cells is schematically shown in **Figure 11-2**. However, although the sequence of events is well characterized and although a Nobel prize was awarded in 1938 to Heymans, a Belgian physiologist, for showing that the carotid body function is responsible for hypoxic hyperventilation, the actual mechanism of oxygen sensing is not clear. Both mitochondrial and membrane models for oxygen sensing have been presented, and several intracellular messenger systems of carotid body glomus cells respond to hypoxia. In the mitochondrial model, the flux of electrons to the final electron acceptor oxygen is reduced, whereby the mitochondrial membrane is depolarized leading to calcium efflux, which stimulates the secretion of excitatory neurotransmitters, and leads to increased activity of afferent nerve fibers (Lahiri et al., 2006). In the membrane model, hypoxia decreases the flux of ions via plasma membrane potassium channels thereby depolarizing the cells, and leading to the influx of calcium from extracellular space (Lahiri et al., 2006). Experimental evidence supporting both models is available. Several inhibitors of mitochondrial function also inhibit the hypoxia response of glomus cells (Lahiri et al., 2001, 2006). The mitochondrial cytochrome a_3 has been suggested as a primary oxygen sensor in the glomus cell (Wilson et al., 1994). On the other hand, extramitochondrial or cell membrane-associated cytochromes have also been implied as primary oxygen sensors (Porwol et al., 2001). In both the mitochondrial and membrane models, redox changes and ROS are involved (Lahiri et al., 2001; Porwol et al., 2001; Lopez-Barneo, 2003), but whereas hypoxia is associated with a decrease in ROS in the case of extramitochondrial control (Porwol et al., 2001), an increase occurs at low oxygen levels in mitochondria (Guzy and Schumaker, 2006). Also, on the basis of present data it appears that both NO and CO can influence the effect of hypoxia on neural discharge from the carotid body glomus cells (Lahiri et al., 2006). Notably, NO influences the oxygen dependency of mitochondrial respiration (Koivisto et al., 1997). In addition, interaction between oxygen-dependent and pH-dependent phenomena occurs in glomus and other excitable cells (Miller et al., 2004; Peers, 2004). It is, furthermore, possible that a globin, neuroglobin, is involved in oxygen sensing, since it has been characterized also in the carotid body (Di Giulio et al., 2006). In sustained hypoxia, it appears that gene expression in the carotid body cells is modified via the HIF-dependent pathway as in other cell types (Fung and Tipoe, 2003; Lopez-Barneo, 2003).

Figure 11-2

A schematic representation of how a decrease in oxygen tension (hypoxia) may affect carotid body glomus cell function. In the mitochondrial model, hypoxia affects either reactive oxygen species (ROS) production or ATP production of mitochondria. Both of these may affect the outward flux of potassium via the potassium channel with the downstream effects shown in the diagram. In the membrane model, the ROS production by membrane-bound molecules (cytochromes) is oxygen sensitive, and thereby affected by hypoxia. Thus, these membrane-bound molecules function as proximal oxygen sensors and cause effects on potassium channels with the downstream effects described in the figure and in the text



5.3 An Example of Other Excitable Cellular Systems: Gill Neuroepithelial Cells

Cells that were classified as neuroepithelial cells were microscopically observed in fish gills in early 1980s (Dunel-Erb et al., 1982). Since breathing rate in fishes is mainly regulated by oxygen, several studies have investigated the possible effects of oxygen on these cells. The neuroepithelial cells share characteristics with both the carotid body glomus cells and lung neuroepithelial cells (Burleson et al., 2006) and have high levels of biogenic amines (Dunel-Erb et al., 1982; Burleson et al., 2006). The branchial neuroepithelial cells are situated in the primary lamellae between water and blood, but appear to have no direct contact with environmental water (Dunel-Erb et al., 1982). Burleson and coworkers (2006) cultured the putative neuroepithelial cells to evaluate whether they had oxygen-sensitive potassium channels, which are a characteristic of oxygen-sensing cells of the carotid body (Donnelly, 1997; 1999; Lopez-Barneo et al., 1999).

The results indicated this to be the case. Notably, the function of oxygen-sensing cells in the gills of fishes have so far been studied only in a couple of species—notably the zebrafish (Jonz et al., 2004) and the channel catfish (Burleson et al., 2006).

6 The Importance of Oxygen Sensing in Neural Function

When the P_{O_2} in the medulla falls below threshold, putative oxygen sensing neurons increase sympathetic activity (Solomon, 2000) triggered by the release of ATP to stimulate an “adaptive increase” in breathing (reviewed by Gourine, 2005). The oxygen-chemosensitive cell of the central nervous system is encompassed in a distributed network of neurons in the brain stem (reviewed by Neubauer and Sunderram, 2004). Central oxygen responsive neurons mediate short- or long-term adaptations to hypoxia, which provide both retaliatory and pre-emptive neuroprotective responses. The increased ventilatory response provides a neuroprotective function by pre-empting energy failure due to a mismatch of oxidative metabolism with energy expenditure. While many of the short-term rapid responses to P_{O_2} levels below threshold can be mediated by local peripheral oxygen-sensitive sensors including the carotid body and effectors, long-term acclimatization to hypoxia requires alterations in sympathetic outflow in the associated brain stem nuclei (reviewed by Guyenet, 2000). Using a chemodenervated rat model to remove afferent input from the carotid body, Roux and coworkers (2000) investigated the direct effects of hypoxia on neurons of the tractus solitarius and ventrolateral medulla and provided evidence that tyrosine hydroxylase (TH) mRNA levels increased in a similar manner to the sham-operated group and some degree of ventilatory acclimatization still occurred, revealing that neurons in these two nuclei acted as oxygen sensors in their own right. Furthermore, neurons in the rostral ventrolateral medulla also demonstrated intrinsic oxygen sensitivity: since cultured neurons demonstrated hypoxia-mediated excitation they have been proposed as putative oxygen sensors (Mazza et al., 2000).

It has been known for some time that TH protein expression is oxygen sensitive and shows a graded response to the duration of hypoxic exposure in the rat medulla. A short 3-day exposure resulted in a 26–50% increase in TH depending on the subpopulation of medullary nuclei examined. In contrast, 14 days of hypoxic exposure resulted in 31–41% increases in TH, after which the level of TH returned to baseline (Schmitt et al., 1993). While oxygen sensors in the medulla appear to regulate the level of TH without input from the carotid body, the hypoxia-induced increase in turnover rate of noradrenaline (NE) requires input from the carotid body (Soulier et al., 1992).

Microdialysate collected from the phrenic nerve to measure oxygen-sensitive neurotransmitter release from the network of brain stem nuclei affecting phrenic nerve output demonstrated that there was a time-dependent change in neurotransmitter release in response to hypoxia. This response was biphasic with an initial decrease in taurine followed by a sustained increase and the initial increase in phrenic nerve activity was consistent with a rapid elevation in the excitatory neurotransmitter glutamate accompanied by a more gradual increase in the inhibitory neurotransmitter GABA (Hoop et al., 1999). Such hypoxia-induced respiratory depression parallels neuronal hypometabolism in brain stem regions involved in respiratory and cardiovascular control in hypoxia-intolerant mammals (LaManna et al., 1996) and in the hypoxia and anoxia-tolerant tropical reef shark, *Hemiscyllium ocellatum* (Mulvey and Renshaw, 2000). However, neuronal hypometabolism in the anoxia-tolerant reef shark is not related to brain failure since the brain energy charge was maintained above critical levels even after a 50 min exposure to anoxia (Renshaw et al., 2002).

Hypoxia induced increases in cerebral blood flow provides a second neuroprotective preemptive defence strategy. Putative oxygen sensors that regulate cerebral blood flow were investigated in rats with bilateral lesions of the rostral ventrolateral medulla. Exposure to a Pa_{O_2} of 36 ± 1 mmHg significantly increased cerebral blood flow in control animals up to 204%, but not in animals with bilateral lesions (Underwood et al., 1994). However, in animals with bilateral lesions there was a significantly lower cerebral blood flow response to hypoxia because of a 50–69% decrease in cerebral vasodilation, demonstrating that

neurons of the rostral ventrolateral medulla have a specific oxygen-sensitive role in controlling vascular tone in response to hypoxia. These changes were not detected in response to hypercapnia (Underwood et al., 1994).

7 Clinical Implications

Asphyxia, sleep apnea, vascular cognitive impairment, stroke, some neurodegenerative diseases, and cardiovascular disease are just a few of the conditions that can result in brain damage as a consequence of reduced oxygen levels. A few minutes of oxygen deprivation is enough to cause neuronal death in the mammalian brain (reviewed by Lutz et al., 2003; Acker and Acker, 2004). This is not so for all vertebrates. The challenge of how to protect vulnerable organs, such as the energetically expensive heart and brain, from hypoxia-induced damage depends on a number of adaptations, including those of oxygen sensing. Many adaptations conferring pronounced hypoxia and/or anoxia tolerance have appeared during vertebrate evolution but they appear to have been retained only by a few species of fish and turtles. Examination of hypoxia-tolerant species has revealed that they can reversibly reprogram gene expression to achieve a "protected phenotype" displaying a suite of retaliatory and pre-emptive mechanisms to forestall cell death (reviewed by Lutz et al., 2003). An understanding of the mechanisms involved in the reversible switch to a protected phenotype may provide an insight into potential intervention strategies that can be used in clinical settings to minimize ischemia-reperfusion injury following stroke and heart attack. Comparative physiology is the nursery ground of several potential treatment strategies for use in clinical settings.

Oxygen levels regulate the pattern of gene expression in health and disease via a master switch, HIF-1 α (reviewed by Nikinmaa and Rees, 2005). While HIF-1 α acts as a ubiquitous transcription factor to increase cell survival during hypoxia and all animals studied so far express HIF-1 α , there are only a few vertebrates that can survive prolonged periods of hypoxia or anoxia. Thus, the ability to change the level of HIF-1 α expression per se does not automatically trigger an expression of a hypoxia- or anoxia-tolerant phenotype. An understanding of why HIF-1 α prolongs survival in some vertebrates and not in others could lead to new treatments, which involve manipulating HIF-1 α levels and its half-life.

Oxygen-regulated gene expression via HIF-1 α as a master regulator results in the upregulation of a number of neuroprotective proteins such as EPO, which can protect neurons from apoptotic cell death via its action on the PI-3-k/AKT pathway (Weishaupt et al., 2004). It has been shown that administration of EPO or its induction by hypoxia significantly reduces infarct size in the mammalian brain (Gassmann et al., 2003) and EPO has recently been used successfully in a number of clinical trials (see reviews by Ehrenreich et al., 2004; Gradin et al., 2004; Ren and Finklestein, 2005). As discussed above, the oxygen sensor pathway is closely interlinked with several other stress pathways; so, preconditioning with a stimulus that causes mild cell injury including exposure to low oxygen levels or exercise (Ameln et al., 2005) activates a suite of protective physiological mechanisms (reviewed by Dirnagl et al., 2003), which confer a naturally protected phenotype. For example, stressors that activate the Hsp family of molecular chaperones not only protect cells from subsequent ischemia and block cell death by apoptosis but may also affect a number of neurodegenerative diseases such as Alzheimer's (reviewed by Franklin et al., 2005). The interaction of the oxygen-sensing pathway with other stress pathways opens up the possibility of cross-protection by targeting key neuroprotective chaperones and transcription factors. This is illustrated by the effect of physiological stressors such as heat (Christians et al., 2002) or exercise (Ameln et al., 2005) on elevating the level of Hsps and/or HIF-1 α , respectively. Understanding how to manipulate these neuroprotective pathways is expected to lead to strategies designed to preempt injury and facilitate functional recovery.

Future therapies may focus on the activation of HIF-1 α and its downstream gene products long enough to gain the neuroprotective benefits but not long enough for HIF to ultimately initiate neuronal death via apoptosis (reviewed by Acker and Acker, 2004). It is also likely that selected gene products, such as neuroprotective globins, that are turned on by intermittent hypoxia (Di Giulio et al., 2006) could be administered to reduce neuronal loss and improve recovery after acute insults due to head trauma, ischemic events, and during chronic diseases such as neurodegenerative diseases of the brain, including the retina.

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