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Mitochondrial Complex III: An essential component of universal oxygen sensing machinery?

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Abstract

Oxygen is necessary for the survival of mammalian cells. In order to maintain adequate cellular oxygenation, mammals have evolved multiple acute and long term adaptive responses to hypoxia. These include hypoxic increases in erythropoiesis, pulmonary vasoconstriction and carotid body neurosecretion. Collectively, these responses help maintain oxygen homeostasis as oxygen levels remain scarce. There are multiple effectors proposed to underlie these diverse responses to hypoxia including PHD2, AMPK, NADPH oxidases, and mitochondrial complex III. Here I propose a model wherein complex III is integral to oxygen sensing in regulating diverse response to hypoxia.

1. Introduction

Multi-cellular organisms have evolved multiple mechanisms to respond to decreased oxygen levels (hypoxia)(Semenza, 2007). Three prominent physiological responses are (1) production of the hormone erythropoietin (EPO) to enhance red blood cell mass and hemoglobin concentration in the blood; (2) pulmonary vascular constriction to shunt blood to better oxygenated regions of the lung; (3) and neurosecretion by the carotid body to increase ventilation(Weir et al., 2005). The latter two responses involve acute oxygen sensing mechanisms resulting in calcium dependent vasoconstriction of pulmonary vessels and neurotransmitter release by the carotid body. By contrast, the former response involves transcriptional upregulation of EPO gene expression in liver and kidney(Ratcliffe et al., 1997). Multiple oxygen sensing mechanisms have been proposed to explain these diverse responses to hypoxia. In this review I propose a model of oxygen sensing whereby mitochondrial complex III is an integral part of oxygen sensing machinery in these diverse responses to hypoxia.

2. Mitochondrial complex III generates superoxide

The electron transport chain (ETC) is the major site of non-enzymatic superoxide formation within mitochondria(Turrens, 2003). The ETC is made up of four multi-protein complexes

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(I-IV) embedded in the inner membrane. Complex I and II oxidize NADH and FADH₂ respectively, transferring the resulting electrons to ubiquinol, which carries electrons to Complex III. Complex III shunts the electrons across the intermembrane space to cytochrome *c*, which brings electrons to complex IV. Complex IV then uses the electrons to reduce oxygen to water. Mitochondrial complexes I, II, and III generate superoxide (Murphy, 2009). Complex I and II generate ROS within the mitochondrial matrix while complex III generates ROS into either the matrix or the intermembrane space (Brand, 2010; Muller et al., 2004). Superoxide generated in the intermembrane space can escape into the cytoplasm through voltage-dependent anion channels (Han et al., 2003).

Mitochondrial complex III generates superoxide during the ubiquinone (Q)-cycle (Sun and Trumpower, 2003). The Q-cycle involves the transfer of two electrons to ubiquinone from complex I and complex II resulting in the reduction of ubiquinone to ubiquinol (QH₂). The subsequent oxidation of ubiquinol at complex III requires the donation of those two electrons to the single electron carrier cytochrome *c*. The first electron transfer at complex III is to the Reiske iron-sulfur center protein (RISP). This electron is then transferred to cytochrome *c*₁ and subsequently to cytochrome *c*. This one electron transfer from ubiquinol results in the unstable radical ubisemiquinone (Q[•]) which can donate its unpaired electron to oxygen to generate superoxide within the Q-cycle. Under most circumstances however, the unpaired electron of ubisemiquinone is transferred to the two heme groups of cytochrome *b* (Heme *b_L* and Heme *b_H*). The two hemes have different electron affinities because they are located in different polypeptide environments. Heme *b_L* is located closer to the intermembrane space and has a lower affinity for electrons than Heme *b_H* which is located closer to the matrix side. Ubisemiquinone transfers its electron to *b_L* to form ubiquinone. Heme *b_L* in turn donates an electron to Heme *b_H*, which subsequently reduces another molecule of ubiquinone, forming ubisemiquinone. The Q-cycle at this stage is only half complete as only one electron from ubiquinol has been transferred to cytochrome *c*. After a second round of the Q-cycle, two molecules of ubiquinol have been oxidized on the intermembrane side of the inner membrane, two molecules of cytochrome *c* have been reduced, and one molecule of ubiquinone has been reduced on the matrix side of the inner membrane.

Much of the information regarding superoxide generation within the Q-cycle comes from the use of mitochondrial inhibitors (Muller et al., 2004; Turrens et al., 1985). The generation of superoxide by ubisemiquinone can be prevented by stigmatellin, which inhibits the oxidation of ubiquinol to ubisemiquinone by preventing electron flux to the iron-sulfur protein (Muller et al., 2004). In fact, any strategy to prevent electron flux to iron-sulfur protein, cytochrome *c*₁ or cytochrome *c* will prevent the oxidation of ubiquinol to ubisemiquinone thereby diminishing the generation of superoxide. Antimycin A increases the generation of superoxide within the Q-cycle by preventing the oxidation of Heme *b_H* (Turrens et al., 1985). This increases the steady state concentration of ubisemiquinone (Q[•]) resulting in an increase in the production of superoxide.

3. Hypoxia increases erythropoiesis

One important mechanism required for organismal maintenance of oxygen homeostasis in higher organisms is the ability to increase the capacity for systemic oxygen delivery as oxygen levels decrease (Semenza, 1994, 2007). This requires an increase in erythropoiesis, the generation of red blood cells. Erythropoiesis is induced under conditions of decreased oxygen availability through an increase in expression of the erythropoietin (EPO) gene (Ratcliffe et al., 1997). EPO gene is a transcriptional target of the Hypoxia-Inducible Factor (HIF) (Wang and Semenza, 1993b). In fact early examination of the regulation of erythropoietin in response to oxygen led to the discovery of the transcription factor

HIF-1 (Semenza and Wang, 1992). Recent studies indicate that in vivo the EPO gene is activated primarily by HIF-2 (Gruber et al., 2007; Rankin et al., 2007).

HIF-1 is a heterodimer consisting of two basic helix loop-helix/PAS proteins, HIF α and the aryl hydrocarbon nuclear translocator (ARNT or HIF-1 β) (Wang et al., 1995). To date three HIF α isoforms have been described HIF-1 α , HIF-2 α , and HIF-3 α (Gu et al., 1998; Tian et al., 1997; Wang and Semenza, 1993a). HIF-1 α and HIF-2 α are transcriptionally active and their target genes have been described to be both overlapping and distinct. The function of HIF-3 α is less well defined, however it is thought to be an antagonist for HIF-1 α and HIF-2 α mediated transcription. Both HIF α and ARNT protein subunits are expressed ubiquitously, but the stability of each protein is differentially regulated by oxygen levels. HIF α protein levels are labile at normal oxygen conditions, while HIF β protein levels are constitutively stable (Huang et al., 1998). Oxygen levels regulate the hydroxylation of two proline residues, 402 and 564, within the oxygen dependent degradation domain (ODDD) of HIF α (Ivan et al., 2001; Jaakkola et al., 2001; Masson et al., 2001). This hydroxylation reaction is catalyzed by the prolyl hydroxylase domain 2 protein (PHD2) (Bruick and McKnight, 2001; Epstein et al., 2001; Huang et al., 2002). PHD2 requires Fe²⁺, oxygen, and 2-oxoglutarate to catalyze the hydroxylation reaction. Hydroxylated prolines serve as binding sites for the von Hippel-Lindau protein (pVHL), the substrate recognition component of the VBC-CUL-2 E3 ubiquitin ligase complex (Hon et al., 2002; Min et al., 2002). Once bound, pVHL ligates HIF α with ubiquitin thereby targeting it for proteasomal degradation. HIF α also contains two transactivation domains. The amino-terminal transactivation domain (N-TAD) is located within the ODDD. The carboxy-terminus transactivation domain (C-TAD) contains an asparagine residue (Asn803) that is hydroxylated by Factor Inhibiting HIF (FIH) (Hewitson et al., 2002; Lando et al., 2002; Mahon et al., 2001). This reaction takes place under normoxic conditions (21% O₂) and it inhibits the transactivation potential of HIF α by preventing its interaction with the co-activator CBP/p300. When oxygen levels decrease below 5% O₂, HIF α protein is stabilized due to a lack of proline hydroxylation. HIF α then translocates to the nucleus and dimerizes with HIF β and binds to HIF response elements (HRE) located throughout the genome. The absence of a hydroxyl group on Asn803 allows HIF to associate with the co-activator CBP/p300 to facilitate the transcription of various target genes. Therefore, regulation of HIF activity is tightly controlled by the hydroxylation of various amino acids.

It is clear that the PHDs are the most proximal molecules regulating the stabilization of the HIF α protein (Schofield and Ratcliffe, 2004). The original and simplest model of oxygen sensing places PHD2 as the direct sensors of oxygen levels. PHD2 requires oxygen for function. Thus, as oxygen availability decreases, PHDs are deprived of this crucial cofactor and become unable to hydroxylate HIF. As the result, the HIF- α protein is not recognized by pVHL and is not degraded. However, while elegantly simple, this model may not by itself explain the oxygen-dependent stabilization of the HIF- α protein. HIF α protein stabilization increases in an exponential fashion as oxygen levels decrease to anoxia (Jiang et al., 1996). By contrast, the PHD2 activity decreases in a linear fashion as oxygen levels decrease to anoxia (Koivunen et al., 2004). During anoxia, oxygen becomes a limiting substrate for hydroxylation. However, the rate of hydroxylation of HIF- α protein in the hypoxic range is likely dependent on the availability of the required co-factors iron and 2-oxoglutarate (Pan et al., 2007). Thus an integrated oxygen sensing model has to account for how rate limiting is oxygen and other co-factors for HIF-1 α protein stabilization over the hypoxic range.

We propose that an increase in mitochondrial complex III-generated ROS in conjunction with the decrease in PHD2 activity during hypoxia prevents hydroxylation of HIF α protein. Early evidence using mitochondrial inhibitors that prevent or maintain complex III-generated ROS suggested the involvement of this electron transport chain complex in

regulation of HIFs (Chandel et al., 1998). Antimycin A indeed maintains the hypoxic increase in ROS production and stabilization of HIF-1 α protein while stigmatellin prevents hypoxic increase in ROS and stabilization of HIF-1 α protein (Chandel et al., 2000). Genetic evidence to support these data comes from the observations that loss of RISP or cytochrome *c* prevents increase in ROS and stabilization of HIF-1 α protein (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005). Furthermore, cells which contain a deletion of the cytochrome *b* gene are respiratory deficient, yet are capable of generating ROS at the Q_o site of complex III and stabilizing HIF-1 α during hypoxia (Bell et al., 2007). Targeting of RISP in the cytochrome *b*-deficient cybrids with RNAi abolished ROS generation and hypoxic stabilization of HIF-1 α protein. These experiments demonstrate that it is not the ability of mitochondria to consume oxygen rather it is their ability to produce ROS at mitochondrial complex III which is crucial for hypoxic stabilization of HIF- α protein.

Another regulator of HIFs are the NADPH oxidase (NOX) family members. Five Nox isoforms (Nox1 to 5) form the basis of distinct NADPH oxidases (Petry et al., 2010). The prototypic NADPH oxidase is composed of two membrane bound components consisting of a catalytic Nox2 subunit (also known as gp91^{phox}) and a p22^{phox} subunit (Babior, 1999). The activation of this catalytic core relies on association with several cytosolic proteins, p67^{PHOX}, p47^{PHOX}, p40^{PHOX}, and rac 1 or 2. Activation of the oxidase occurs when the cytosolic components migrate to the cell membrane so that the complete oxidase can assemble. The notable exception is Nox4 which does not require p47^{phox}, p67^{phox}, or Rac for activation and is bound to intracellular organelles such as the endoplasmic reticulum and mitochondria (Block et al., 2009; Wu et al., 2010). A consequence of HIF-1 activation is the induction of NADPH oxidase family members 4 (NOX4) which would further amplify ROS levels and the activation of HIFs (Diebold et al., 2010). Hypoxic increases in NOX1 activity also can further stimulate HIF-1 transcriptional activity (Goyal et al., 2004). Moreover, non-hypoxic induction of HIF-1 by thrombin is mediated by activation of a p22^{phox}-containing NADPH oxidase complex (Görlach et al., 2001). Thus, NOX family members are likely to be important contributors to the oxygen sensing machinery by amplifying the hypoxic ROS signaling.

4. Hypoxia increases pulmonary vasoconstriction

While erythropoiesis takes hours to days to occur, the acute response to hypoxia occurs within seconds and involves vasoconstriction of the pulmonary circulation (Weir et al., 2005). In response to hypoxia, the arterial pressure of the pulmonary artery increases, allowing for improved gas exchange by diverting blood from poorly oxygenated regions of the lung. Although the pulmonary endothelium can amplify this response, isolated pulmonary arterial smooth muscle cells (PASMCs) contract during hypoxia, indicating that the hypoxic pulmonary vasoconstriction (HPV) response is initiated in PASMCs (Mark Evans and Ward, 2009). Hypoxia triggers contraction of PASMCs through an increase in cytosolic calcium from both intracellular and extracellular stores (Ward et al., 2004). An increase in cytosolic calcium causes calmodulin-mediated activation of myosin light chain kinase, actin-myosin interaction, and contraction. However, the exact mechanism of this calcium elevation is not fully understood. How decreases in oxygen are detected and coupled to an increase in calcium is being intensely investigated currently.

Primary evidence for the involvement of mitochondrial complex III in regulating hypoxic calcium increase and subsequent contraction of PASMCs comes from Schumacker and colleagues who demonstrated that HPV in isolated rat lungs or contraction in PASMCs during hypoxia is blocked by diphenyleneiodonium (DPI), myxothiazol, or rotenone (Waypa et al., 2001). All three mitochondrial inhibitors block electron transport chain upstream from the ubiquinone site of superoxide production within complex III. DPI, rotenone, and

myxothiazol also attenuated the increase in calcium in response to hypoxia without affecting the increase in calcium due to angiotensin II during normoxia (Waypa et al., 2006; Waypa et al., 2002). By contrast, antimycin A, which inhibits mitochondrial electron transport chain downstream from the ubisemiquinone site of superoxide production, maintains HPV and hypoxia-induced calcium signaling. Antioxidants abolished pulmonary vasoconstriction in isolated lungs and contraction in PSMCs during hypoxia (Waypa et al., 2001). H₂O₂ was sufficient to invoke contraction in PSMCs and increase calcium during normoxia. Adenovirus transfection of PSMCs with catalase prevented the increase in calcium during hypoxia or H₂O₂ during normoxia (Waypa et al., 2006; Waypa et al., 2002). Multiple other investigators have corroborated these findings (Leach et al., 2001; Rathore et al., 2008; Weissmann et al., 2003). Recently, Schumacker and colleagues have targeted redox sensitive GFP (roGFP) to different mitochondrial compartments to spatially analyze where the ROS levels are increased (Waypa et al., 2010). Interestingly, hypoxia simultaneously increases roGFP oxidation in the mitochondrial intermembrane space and a decrease in roGFP oxidation. An important step to further validate these findings would be to genetically manipulate complex III to demonstrate it indeed is regulating the hypoxic increase in ROS within the intermembrane space.

ROS required for HPV have also been suggested to result from the activation of NADPH oxidase in hypoxic PSMCs (Grimminger et al., 1995; Weissmann et al., 2000). Mice lacking p47^{PHOX} display reduced HPV (Weissmann et al., 2006). Furthermore, PSMCs isolated from p47^{PHOX} null mice or wild-type cells treated with the pan NOX inhibitor apocynin both display diminished hypoxic increase in intracellular ROS, calcium and contraction (Rathore et al., 2008). Interestingly, the increase in NOX activity observed in PSMCs during hypoxia is dependent on mitochondrial ROS generation (Rathore et al., 2008). Antioxidants and mitochondrial inhibitors that attenuate complex III ROS both prevented hypoxic induction of NADPH oxidase activity. The link between the mitochondrial complex III ROS and NADPH oxidase (s) is protein kinase C epsilon (PKCε). Pharmacologic or genetic ablation of PKCε attenuated hypoxia induced increase in NADPH oxidase activity, ROS, calcium, and contraction in PSMCs (Rathore et al., 2008). Furthermore, the acute HPV is inhibited in isolated lungs from PKCε null mice. Mitochondrial inhibitors rotenone and myxothiazol both prevented the acute hypoxic increase in PKCε activity in mouse pulmonary arteries suggesting that mitochondrial ROS are upstream of PKCε. Thus, hypoxia is sensed in the mitochondria of PSMCs and ROS produced at complex III and NADPH oxidases act synergistically to elevate cytosolic calcium, inducing pulmonary vasoconstriction during hypoxia (Wang and Zheng, 2010). It will be important to utilize the various NOX family member knockouts to determine which NOX is responsible for HPV.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is another proposed regulator of oxygen sensing in PSMCs (Evans, 2006a). AMPK is a heterotrimeric serine/threonine kinase complex consisting of a catalytic α subunit and two regulatory β and γ subunits (Kahn et al., 2005). AMPK is ubiquitously expressed and functions as an intracellular energy sensor by facilitating ATP production and suppressing unnecessary ATP use in energy-stressed cells (Hardie, 2007). To date, conditions that elevate intracellular AMP or decrease ATP levels are known to activate AMPK through the allosteric binding of AMP, which allows AMPK to sense cellular [AMP]/[ATP] ratios. Full activation of AMPK requires specific phosphorylation (Thr172) within the activation loop of the catalytic domain of the α-subunit by upstream kinases, including LKB1, a serine/threonine protein kinase and tumor suppressor (Hawley et al., 2003; Shaw et al., 2004; Woods et al., 2003). Mammalian Ca²⁺/calmodulin-dependent kinase kinase beta (CaMKKβ) has also been identified as an AMPK kinase (Hawley et al., 2005). AMPK phosphorylates diverse targets, many of which are directly involved in controlling cellular energy metabolism, such as acetyl-CoA

carboxylase (ACC) and glycogen synthase (Hardie, 2007). The net effect of AMPK activation is to inhibit lipid and glycogen synthesis concomitant with the activation of fatty acid oxidation and glycolysis. Thus, AMPK switches the cell from energy-storing to releasing energy for use under conditions where ATP is limiting.

Pharmacologic activation of AMPK by AICAR or hypoxia in PSMCs evokes an increase in intracellular calcium from ryanodine-sensitive calcium channels in the sarcoplasmic reticulum as well as HPV in isolated lungs (Evans et al., 2005). Furthermore, the AMPK antagonist compound C prevents HPV (Robertson et al., 2008). Compound C can potentially affect other pathways therefore these studies need to be confirmed with knockout mice deficient in AMPK (Emerling et al., 2007). However, a paradox that needs to be resolved with respect to AMPK activation in PSMCs is that hypoxia (1-2 % O₂) does not elicit changes in AMP in most cell types (Emerling et al., 2009). Oxygen is not rate limiting to cytochrome *c* oxidase, the main complex in the mitochondria that utilizes oxygen, in virtually every metazoans until 0.5% O₂ (Murphy and Brand, 1987). *Paracoccus Denitrificans*, a bacterium with a similar mitochondrial electron transport chain to modern mitochondria, also has a cytochrome *c* oxidase with a high affinity towards oxygen (Ludwig and Gibson, 1981). However, some investigators have argued that PSMCs might have a specialized cytochrome *c* oxidase that has a low affinity for oxygen thus allowing AMP/ATP ratio to increase at much higher oxygen levels (Evans, 2006b). This intriguing hypothesis needs to be experimentally confirmed. An alternative explanation is that AMPK activation is dependent on mitochondrial complex III generated ROS. Recently, we reported that hypoxic activation in cancer cells is dependent on mitochondrial complex III ROS independent of AMP/ATP ratio (Emerling et al., 2009). In accordance with other reports, we found no increase in AMP/ATP ratio during hypoxia (Liu et al., 2006). Furthermore, genetic ablation of AMPK did not prevent hypoxic activation of HIF-1 (Emerling et al., 2007). Based on these observations we suggest that mitochondrial complex III, NADPH oxidase and AMPK collectively fine tune the oxygen sensing machinery in PSMCs to regulate hypoxic pulmonary vasoconstriction.

5. Hypoxia increases carotid body nerve firing

Arterial oxygen pressure <60 mm Hg in the systemic blood results in neurosecretion by carotid body type I cells (glomus cells), which activate afferent sensory fibers of the sinus nerve to stimulate the brainstem respiratory centers and provoke an increase in ventilation (López-Barneo et al., 2008). The molecular mechanism underlying this phenomenon is that hypoxia elicits inhibition of potassium channels triggering membrane depolarization resulting in calcium influx, neurosecretion and carotid body nerve firing (Weir et al., 2005). There are multiple oxygen sensitive channels in the glomus cells (Buckler, 1997; López-Barneo et al., 1988; Peers and Wyatt, 2007). Two major questions unresolved in the field remain the nature of the potassium channels and the nature of the oxygen sensing machinery. Multiple mechanisms have been proposed to explain oxygen sensing in the carotid body including NADPH oxidases, heme oxygenase 2 (HO-2), mitochondria, and AMPK (Ward, 2008). Interestingly, the PHD2-HIF-1 axis is unlikely to play a role in acute oxygen sensing in the carotid body since the pharmacologic inhibition of PHD2 or HIF1^{+/−} mice both display intact acute hypoxic response in the carotid body slices (Kline et al., 2002; López-Barneo et al., 2008). In the next section I review the evidence to support these different putative sensors.

NADPH oxidase was first proposed to be an O₂ sensor in carotid body by Acker and colleagues based on their observations that DPI could suppress hypoxic excitation of the carotid body afferent sensory nerves (Cross et al., 1990). However, DPI inhibits NOX family members, mitochondrial complex I, and ion channels. Moreover, genetic loss of

NOX2 or the p47phox subunit in mice does not alter carotid body nerve firing (Archer et al., 1999; He et al., 2002; Roy et al., 2000). It is possible that multiple NOX family members could contribute to hypoxic increase in carotid body neurosecretion. As all five NOX family members utilize the p22 phox protein to generate superoxide, a critical experiment to test definitively the involvement of NOX family members would be to examine carotid body nerve firing in response to hypoxia in the p22 phox subunit null mice.

HO-2 is a constitutively active enzyme found in glomus cells which utilizes oxygen to convert heme into iron, biliverdin, and carbon monoxide (CO) (Maines, 2005). Numerous studies have highlighted the importance of CO as a signaling molecule over the past two decades (Piantadosi, 2008). Kemp and colleagues in their elegant study demonstrated that RNAi of HO-2 abolishes the hypoxic sensitivity of maxi-K⁺ channels and HO-2 co-immunoprecipitates with this channel in HEK-293 cells (Williams et al., 2004). However, genetic ablation of HO-2 in mice does not alter neurosecretion of glomus cells upon exposure to hypoxia (Ortega-Sáenz et al., 2006). Importantly, HO-2 loss was not compensated with an increase in HO-1 expression in carotid body.

Mitochondria have been traditionally proposed as site of oxygen sensing within glomus cells for carotid body nerve firing (Mills and Jöbsis, 1970, 1972). Mitochondrial electron transport chain inhibitors increase glomus cell neurosecretion and the afferent activity of the sinus nerve under normoxia and further amplify the hypoxic response (Duchen and Biscoe, 1992; Ortega-Sáenz et al., 2003; Wyatt and Buckler, 2004). Furthermore, it has been proposed that the glomus cell cytochrome c oxidase has an unusually high K_m for oxygen (Mills and Jöbsis, 1970). Therefore, as oxygen levels diminish, cytochrome c oxidase activity would diminish resulting in a decrease in intracellular ATP and a rise in the AMP/ATP ratio. This would result in activation of AMPK. Recent studies indicate that pharmacologic inhibition of AMPK inhibits the hypoxic increase in intracellular calcium in isolated carotid body glomus cells and the sensory afferent discharge (Wyatt et al., 2007). Consistent with this data is that mitochondrial inhibitors, which increase the AMP/ATP ratio, thus activate AMPK trigger glomus cell neurosecretion under normoxia. It would be important to inhibit AMPK activation in the presence of mitochondria inhibitors. Furthermore, the role of AMPK needs to be genetically corroborated using knockout mice. The other key point that needs to be addressed is how AMPK is activated in the glomus cells.

Aside from AMP/ATP ratio, an increase in ROS or calcium can also activate AMPK. Hypoxia also increases ROS in carotid body (Yamamoto et al., 2006). Interestingly, the effects of mitochondrial inhibitors and hypoxia are additive with respect to glomus cell neurosecretion rate (Ortega-Sáenz et al., 2003). The one exception is rotenone which prevents complex III ROS generation. However, rotenone could have other off target effects. It would be informative to know the effects of the complex III inhibitor stigmatellin on catecholamine secretion by glomus cells during hypoxia. Stigmatellin is more effective than myxothiazol in preventing complex III superoxide generation (Muller et al., 2004). Since electron transport inhibitors themselves affect neurosecretion, it would be more advantageous to examine the effect of targeted mitochondrial targeted antioxidants (i.e. MITOQ) on hypoxic sensitivity of the glomus cells. Schultz and colleagues recently demonstrated that adenovirus-mediated gene transfer of SOD1 or SOD2 decreases the carotid body chemoreceptor activity under baseline conditions and in response to hypoxia in rabbits with chronic heart failure (Ding et al., 2009; Ding et al., 2010). These authors implicate both NADPH oxidase family members and mitochondria as contributor of ROS generation in the carotid body. In summary, the exact role of mitochondria remains elusive with respect to hypoxic increase in glomus cell secretion. I speculate that a combination of

mitochondria, NADPH oxidase and AMPK are all likely involved in the regulation of hypoxic increase in carotid body nerve firing.

6. Conclusion

Much progress has been made in understanding how cells sense oxygen and transduce adaptive signaling cascades. Yet, there the exact nature of oxygen sensing is not fully understood in different responses to hypoxia. The emerging picture in regulation of hypoxic gene expression is dependent on hypoxic inhibition of PHD2 activity coupled with an increase in mitochondrial complex III ROS generation which is further amplified by NOX family members (Figure 1). Hypoxic pulmonary vasoconstriction is dependent on mitochondrial complex III ROS generation and NOX family members coupled with AMPK activation (Figure 1). It is not clear the relative importance of mitochondria, AMPK, and NOX family members. The advent of conditional genetic knockouts in these pathways will further elucidate the mechanisms of oxygen sensing for both the acute and prolonged response to hypoxia.

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Figure 1. Oxygen sensing mechanisms during acute and prolonged hypoxia

Acute oxygen sensing is important for hypoxic pulmonary vasoconstriction (HPV) and hypoxic neurosecretion by the carotid body (CB). These two responses are dependent on an increase in intracellular calcium. Mitochondrial complex III generated ROS are critical for increased intracellular calcium levels which trigger HPV. This signal is amplified by activation of NOX family members. AMPK is also important for the rise in calcium during HPV. However, it is not clear whether an increase in mitochondrial ROS or AMP/ATP ratio is critical for AMPK activation during hypoxia. Presently, the role of mitochondrial ROS or NOX family members is not clarified for CB neurosecretion during hypoxia. However, there is data to support that AMPK activation is necessary for CB neurosecretion during hypoxia. The prolonged response to hypoxia triggers the activation of the transcription factor HIFs which are composed of two subunits, an oxygen sensitive HIF- α and HIF- β . HIF- α is hydroxylated at two different proline residues under normoxia by PHD2. The hydroxylation of proline residues serves as a recognition motif for pVHL. The binding of pVHL targets the HIF- α protein for ubiquitin mediated degradation. Under hypoxia, the hydroxylation of proline is diminished, which allows for the protein to be stabilized and bind to HIF-1 β as well as p300/CBP to allow HIF dependent transcription of genes such as EPO which increases red blood cell proliferation and maturation to increase oxygen carry capacity in blood. Present data suggests that hypoxia increases mitochondrial complex III ROS production, diminishing the hydroxylation of the HIF- α protein. NOX family members are also transcriptionally induced by HIF-1, further amplifying the ROS signal.