Forum Review Article

How does the heart sense changes in oxygen tension – a role for ion channels?

Livia C. Hool

School of Anatomy, Physiology and Human Biology, The University of
Western Australia, Crawley, Australia

Address for correspondence:

Professor Livia Hool

Physiology M311

School of Anatomy, Physiology and Human Biology

The University of Western Australia

Stirling Highway

Crawley, WA, 6009,

AUSTRALIA.

Telephone: 61 8 6488 3307

Facsimile: 61 8 6488 1025

E-mail: livia.hool@uwa.edu.au

Running title: Oxygen sensing in the heart

9 figures, 133 references

Abstract

Significance: Oxygen occupies a key role in cellular metabolism and function. Oxygen delivery to cells is crucial and a lack of oxygen such as occurs during myocardial infarction can be lethal. Cells must therefore be able to respond to changes in oxygen tension. **Recent advances:** Since the first studies examining the acute cellular effect of hypoxia on activation of transmitter release from glomus or type I chemoreceptor cells, it is now known that virtually all cells are able to respond to changes in oxygen tension.

Critical Issues: Despite advances made in characterising hypoxic responses, the identity of the “oxygen sensor” remains debated. Recently, more evidence has evolved as to how cardiac myocytes sense acute changes in oxygen.

This review will examine the available evidence in support of acute oxygen sensing mechanisms providing a brief historical perspective and then more detailed insight into the heart and the role of cardiac ion channels in hypoxic responses. **Future Direction:** Further understanding these cellular processes should lead to interventions that assist in preventing the deleterious effects of acute changes in oxygen tension such as alterations in contractile function and cardiac arrhythmia.

I. Introduction

All aerobic organisms require O_2 for life. Cells must therefore possess a mechanism for sensing changes in O_2 tension and eliciting a response. It is assumed that acute changes in O_2 tension will result in adaptive responses that are designed to restore O_2 to tissue and maintain normal cellular function. This is true of cardiorespiratory reflexes in the brain that respond rapidly to discharge levels from afferent chemosensory fibres in the carotid body as a result of alterations in blood levels of O_2 , CO_2 or pH. In addition, acute changes in hypoxia may also contribute to maintaining cell survival until normoxia is restored. Prolonged periods of hypoxia however lead to more distinct remodelling of cells including changes in transcription and translation of proteins that can contribute to pathophysiological states such as dementia (1-3), heart failure (4, 5), vascular disease (6-8) and cancers (9, 10).

Cardiovascular disease remains the leading cause of death in the western world and the majority of deaths occur as sudden cardiac deaths (11). An acute change in cellular oxygen tension represents a trigger for cardiac arrhythmia where an appropriate substrate such as myocardial infarction or a defect in a gene encoding an ion channel exists (12, 13). Although significant advancement has been made in understanding how changes in O_2 tension alter the function of glomus cells of the carotid body (14, 15), and the pulmonary (16) and renal vasculature (17, 18), the identity of the “oxygen sensor” remains controversial. This is particularly pertinent with respect to the heart since it is well recognised that this organ is critically dependent on O_2 for normal excitation and contraction. A great deal has been studied and written about how the heart responds to a hypoxic environment. Despite this, it is still

not clear how cardiac myocytes sense changes in O₂ tension. That is, the mechanism/s by which the cell detects acute changes in oxygen tension and translates this into a physiological response. Over the past 15-20 years a number of mechanisms have been proposed for oxygen sensing in the heart. This review will revisit the early hypotheses of oxygen sensing with reference to ion channels and argue the evidence for and against oxygen sensing sites in cardiac myocytes with recent data as support for the arguments. The review will examine the mechanisms for acute oxygen sensing only. That is, physiological responses that occur within seconds of exposure to hypoxia and are not prolonged for hours or days. Ion channels are capable of responding very rapidly to changes in the cell's environment, and rapid changes in oxygen tension can represent acute triggers for sudden cardiac death. Since the mechanism for oxygen sensing is largely unresolved in cardiac tissue, some evidence for mechanisms of oxygen sensing will be drawn from experimental data from other systems. Therefore the review will initially provide some background to mechanisms that have been debated in other systems such as a role for a heme protein in hypoxic sensing or hydroxylation of a protein with reference to ion channels. Possible direct effects of oxygen and carbon monoxide on ion channels will be discussed followed by the evidence for indirect effects of hypoxia via alterations in cellular reactive oxygen species. These include a role for the reactive oxygen species generating mechanisms NAD(P)H-oxidase and mitochondria. Finally the implications for acute hypoxia on the cardiac action potential are discussed.

II. Oxygen sensing mechanisms

A. A heme protein or hydroxylation of a protein?

For some time it was thought that a reduction in binding of O₂ to a heme protein initiated the synthesis of erythropoietin in the kidney and an increase in red blood cell production (19). This was based on the observation that rats injected with cobalt chloride exhibited polycythemia and increased erythropoietin production. It was concluded that cobalt bound to heme and mimicked hypoxia by changing the protein to a deoxy configuration. In addition, it was thought that the posttranslational stabilization of the hypoxia-inducible factor-1 (HIF-1) α subunit during hypoxia involved a heme protein. HIF-1 binds to DNA sequences within hypoxia response elements as a basic helix-loop-helix PAS heterodimer and appears to be involved in a wide range of cellular responses involving DNA binding and transcription of a number of genes during hypoxia. Some of these include glucose transporters, glycolytic enzymes, vascular endothelial growth factor, erythropoietin, inducible nitric oxide synthase (NOS II), and insulin-like growth factor 2 (IGF-2) (20).

However, the induction of erythropoietin and the stabilization of HIF-1 do not involve a heme protein. Prolyl hydroxylation at a conserved core LXXLAP motif of the oxygen degradation domain of HIF-1 results in proteasomal degradation of the HIF-1 α subunit (21). The prolyl hydroxylase is a 2-oxoglutarate-dependent dioxygenase that has an absolute requirement for dioxygen as co-substrate. In addition, the prolyl hydroxylation requires iron as a co-factor. This accounts for the effect of transition metals such as cobalt, manganese and nickel, and iron chelators such as desferrioxamine on HIF-1 induction. HIF-1 α protein levels are induced in an organ-specific manner with expression in the mouse heart clearly detectable after 1 hour of exposure to

hypoxia (22). The effects of HIF-1 on the regulation of cellular transcription typically occur over many hours. However the hydroxylation reaction (and degradation of HIF-1 α occurs much more rapidly. Therefore prolyl hydroxylase serves as an excellent candidate for acute O₂-sensing.

The onset of hydroxylation of prolines within HIF-1 is a rapid reaction. An involvement of protein hydroxylation in the regulation of ion channel function during hypoxia is not well characterised. However hydroxylated residues may be important determinants of channel function since removal of hydroxyl groups in the pore forming region of the α_3 subunit of the human glycine receptor is crucial to receptor channel inactivation (23). In addition, hydroxylated amino acids form the narrow region of the pore of the acetylcholine receptor channel and a hydroxylated serine is a main determinant of channel conductance (24). It was recently identified that the transient receptor potential channel TRPA1 is hydroxylated by HIF during normoxia in *Drosophila Melanogaster* leading to reduced channel activity and internalization of the channel protein (25). During hypoxia the channel becomes activated and internalization is reversed. TRPA1 channels are not expressed in the heart. However, the channels can influence cardiovascular responses through effects on the vasculature (26). Despite the rapid nature of the prolyl hydroxylation reaction, it does not appear to play a significant role in regulation of cardiac function during acute hypoxia.

A heme protein has been implicated in hypoxia responses and in the regulation of maxiK channels in the carotid body (27, 28) and hypoxic pulmonary vasoconstriction (29). Inhibition of maxiK channels occurred in excised patches leading to the conclusion that the response (and location of

the heme O₂ sensor) was membrane-delimited. The protein responsible for sensing the change in oxygen was identified as a heme oxygenase-2 (30). The role of heme oxygenase-1 and heme oxygenase-2, both of which are expressed in the heart is still being fully elucidated (31). Heme oxygenase-1 over expressing mice are protected against ischemia reperfusion injury and develop less remodelling in the heart (32) while heterozygote knockout mice have larger infarcts after ischemia reperfusion (33). **Heme oxygenase-2 may partner with biliverdin reductase to prevent β -adrenergic receptor induced apoptosis in the heart (34). However these responses occur over many hours to days and are adaptive responses to chronic hypoxia.**

With respect to rapid responses, a direct binding of heme oxygenase to the Ca²⁺ /calmodulin-dependent protein kinase II-specific motif on the C terminal end of the L-type Ca²⁺ channel has been implicated in hypoxic regulation of the channel in the heart (35). The degradation of heme leads to the generation of ferrous iron, biliverdin and carbon monoxide (Fig. 1). Significant attention has been placed on understanding the roles of these three products in mediating physiological responses to hypoxia. Ferrous iron induces ferritin expression, that is necessary for iron sequestration. It is recognised that biliverdin has antioxidant and antiapoptotic properties and may also act through the regulation of microRNA's (36). Carbon monoxide mediates cardioprotection and appears to act in a similar manner to nitric oxide. Although conflicting responses have been reported, carbon monoxide can regulate the function of a number of ion channels (37). Much of the data describing effects of carbon monoxide on ion channel function have been derived from expression systems and therefore the net effect on native cell

function or system physiology is still being elucidated. With reference to cardiac ion channels, since the large conductance Ca^{2+} -activated K^{+} channel is expressed in the heart it can be assumed that carbon monoxide activates the channels, while decreasing $\text{K}_{\text{v}2.1}$ channels (38) and L-type Ca^{2+} channels (39). The net effect of carbon monoxide on the action potential is to induce early afterdepolarizations as a result of increasing Na^{+} conductance through the late Na^{+} current (40). Although the 2 pore domain potassium channel TREK-1 is expressed in the heart it has not been demonstrated that carbon monoxide regulates cardiac TREK1 channels.

The mechanism by which carbon monoxide confers changes in ion channel function remains unclear. One mechanism is through activation of signalling molecules such as nitric oxide synthase (that produces nitric oxide) (37), MAP-kinase (41) and by altering mitochondrial production of reactive oxygen species through inhibition of mitochondrial cytochrome oxidase C (39). The alteration in channel function occurs as a result of reactions of signalling molecules with cysteines, threonines or serines on the channel. Since carbon monoxide is produced by a heme oxygenase-2 that can co-localise with BK_{Ca} channels (30), it has been suggested that carbon monoxide can directly alter channel function by binding to a metallo-thiol region within the RCK2 domain of the channel (42, 43). However this region also confers sensitivity to calcium (44) so could be regulated indirectly through an effect of carbon monoxide on calcium regulatory proteins. It has been suggested that carbon monoxide reacts directly with amino acids on the channel protein. However a direct effect of carbon monoxide on channel function must be channel specific in order to explain the variability in responses reported. We

tested whether carbon monoxide can directly alter the function of the L-type Ca^{2+} channel alpha subunit. We expressed the human long NT isoform of $\text{Ca}_v1.2$ in HEK cells, purified the channel protein and then reconstituted the protein in liposomes for functional assessment using patch-clamp technique. Under these conditions we can assess changes in single channel activity in the absence of other regulatory proteins and assess whether carbon monoxide can directly bind and alter channel function. In each patch, we confirm that the current is derived from $\text{Ca}_v1.2$ channels by measuring the magnitude of the current while voltage stepping the channel, calculating open probability (P_o) and assessing the sensitivity of the channel to L-type Ca^{2+} channel antagonist nisoldipine (Fig. 2). The channel protein is also identified as a 240 kDa band on immunoblot when probed with the L-type Ca^{2+} channel antibody (Fig. 2D). We applied the carbon monoxide donor CORM-2 at a concentration of 30 μM directly to the channel incorporated in liposomes in the bath for at least 30 minutes and recorded changes in current. This is a concentration that is effective at inducing vasorelaxation in aortic rings (45) and positive inotropic effects in isolated perfused hearts (46). We found no alteration in P_o or magnitude of current at voltage steps of -200mV to +200mV suggesting that carbon monoxide cannot directly alter channel function (Fig. 3). This is consistent with the finding that nifedipine failed to prevent the increase in systolic pressure and inotropic effects in isolated hearts exposed to carbon monoxide donors (46). It also suggests that the effects of carbon monoxide on L-type Ca^{2+} channel function occur via secondary or indirect modulation of signalling pathways in the heart (rather than direct effects of CO on the channel protein) and is consistent with effects mediated via alterations

in reactive oxygen species (39). The mechanisms for the effect of carbon monoxide on K^+ channels and Na^+ channels in the heart are still being elucidated.

B. The “O₂-sensitive ion channel”

Ion channels have been considered O₂ sensors because their modulation by hypoxia is rapid and occurs in excised membrane patches where cytosolic variables such as second messengers, ATP and Ca^{2+} are thought to be absent. A number of studies have reported modulations in ion channel activity during hypoxia. The first report of an effect of hypoxia on an ion channel was the characterization of O₂-sensitive K^+ channels in glomus or type I cells of the rabbit carotid body (47). O₂-sensitive K^+ channels have been found in a number of neurosecretory cells (48-51). The closure of K^+ channels by hypoxia results in membrane depolarization and Ca^{2+} influx, transmitter release to the innervated organ and activation of afferent sensory fibres.

In neurosecretory cells such as chromaffin cells, hypoxia does not appear to influence Ca^{2+} channels directly. Ca^{2+} influx occurs as a result of membrane depolarization. A similar response is observed in hypoxic pulmonary vasoconstriction as a mechanism to reduce poor ventilation and prevent systemic hypoxemia where atelectasis is present (8, 52). This is not the case however in some pulmonary resistance vessel myocytes where hypoxia increases Ca^{2+} channel conductance (53-55). In fact, ion channel responses to hypoxia appear to vary considerably depending on the cell type (56). Ion channels with PAS domains such as the human ether-a-go-go related (HERG) K^+ channel do not appear to be modulated by hypoxia (57,

58). In arterial smooth muscle cells, hypoxia appears to inhibit L-type Ca^{2+} channels resulting in relaxation of vessels (59, 60). The varied responses by ion channels to changes in O_2 tension has made it difficult to assign a universal O_2 -sensing component to the channel. In addition, there is no experimental or molecular evidence to suggest that the channel protein is capable of directly sensing changes in oxygen tension.

Hypoxia can induce changes in protein function in the absence of cellular metabolic inhibition. Because oxidative phosphorylation and ATP production does not become compromised until oxygen tension falls below 2 mmHg (61, 62) many hypoxic responses occur in the absence of alterations in cellular ATP or adenosine concentrations. ATP-sensitive K^+ channels contribute to repolarization of cardiac myocytes and were originally described as being activated upon depletion of cellular ATP (63). However, it has been suggested that the plasmalemmal channels may also be activated during hypoxia/ischemia in the absence of depletion of ATP and influence cardiac contractility (64). The role of the channels in hypoxic responses remains unclear because myocardial infarct size is unchanged in Kir6.2 null mice suggesting that the channels are not protective against necrotic injury (64). Activation of mitochondrial but not plasmalemmal ATP-sensitive K^+ channels has been implicated in ischemic preconditioning and the mechanisms involve protein kinase C (PKC) (65-68).

Whether hypoxia directly alters the function of ATP-sensitive K^+ channels is unclear. A considerable amount of evidence is now available to suggest that ion channel function can be modified by redox state. The ATP-sensitive K^+ channel may undergo modification of thiol groups during hypoxia

resulting in an alteration in channel function. In support of this, the sulfhydryl agents 5,5'-dithio bis-(2-nitro-benzoic acid) (DTNB) and N-ethylmaleimide have been shown to alter channel function (69). Other cardiac ion channels appear to be modified during hypoxia as a result of altered cellular redox state. Hypoxia has been shown to increase a persistently inactivating Na^+ current in rat ventricular myocytes and central neurones that is postulated to involve redox modification of the channel protein or an associated nearby protein (70-72).

In cardiac myocytes, there is good evidence that hypoxia inhibits the L-type Ca^{2+} channel. This has been demonstrated in HEK 293 cells expressing the α_{1C} subunit of the human cardiac L-type Ca^{2+} channel (35, 73) and in native channels in guinea-pig cardiac myocytes (74, 75) suggesting that there is a requirement for the α_{1C} subunit of the channel in the O_2 -sensing mechanism. In search for the identity of the O_2 -sensing site, Fearon *et al* (76) studied 3 splice variants of the α_{1C} subunit of the human cardiac L-type Ca^{2+} channel. They were able to isolate a 39 amino acid segment of the C terminal domain of the hHt isoform that appeared to be required for inhibition of the channel during hypoxia.

We tested whether the alpha subunit was sufficient for hypoxic inhibition of the human cardiac L-type Ca^{2+} channel. We reconstituted purified human long N terminal (NT) isoform of the $\text{Ca}_v1.2$ in proteoliposomes and tested the effect of altering oxygen tension on single channel activity (Fig. 4). The long NT isoform lacks exon 45 that is proposed to confer oxygen sensitivity (76). Decreasing oxygen tension from room oxygen (150 mmHg) to 15 mmHg had no effect on P_o of the long NT isoform. In addition hypoxia did

not alter the P_o of the short NT hHt isoform that contains exon 45, despite inducing a reversible effect on the native channel in guinea pig myocytes (Fig. 5). These data indicate that the α_{1C} subunit of the channel cannot respond directly to changes in oxygen tension implicating an alternative mechanism or regulatory intermediate protein in hypoxic inhibition of the L-type Ca^{2+} channel.

The L-type Ca^{2+} channel does not possess an O_2 binding domain. However the α_{1C} subunit contains a number of critical thiol groups that can be modified in response to alterations in cellular redox state that occurs during hypoxia. In fact there are approximately 53 cysteine residues on the $Ca_v1.2$ although not all of these will be freely available to react because some are embedded in the lipid bilayer (Fig. 6). Therefore alterations in channel function may occur because of modifications in thiol groups critical to function. Consistent with this, we and others have shown that alterations in cellular production of $O_2^{\cdot-}$ and H_2O_2 can modulate ion channel function (77-80). Redox modification of channel function has been demonstrated in native channels where all channel subunits are present (74, 75, 79, 81) and in the purified $Ca_v1.2$ subunit of the channel reconstituted in proteoliposomes (82). Direct modification of thiol groups on the channel with application of DTNB or oxidised glutathione (GSSG) directly increases P_o while dithiothreitol and reduced glutathione (GSH) decrease P_o (Fig. 7). We have demonstrated that oxidative stress is associated with an increase in channel activity as a result of direct glutathionylation of the $Ca_v1.2$ subunit (82). In addition $Ca_v1.2$ is glutathionylated in human ischemic heart (82). Given that O_2 -sensitivity is not a universal characteristic of ion channels, and ion channels respond

differentially to hypoxia, it appears that ion channels are not oxygen sensors *per se* but effectors responding as a consequence of the signal from the putative sensor. Reactive-oxygen species are capable of acting as signalling molecules. In addition, changes in cellular oxygen tension are associated with alterations in cellular redox state. Therefore, possible candidates for O₂-sensing must also include those that are capable of generating reactive-oxygen species as signals.

C. Reactive-oxygen species as signals

Reactive-oxygen species (ROS) can act as mediators of hypoxic responses. The term ROS generally groups oxygen molecules in different redox states and electronic excitation as well as compounds of oxygen with hydrogen and nitrogen. The production of ROS begins with the reduction of O₂ to superoxide anion (O₂^{•-}). O₂^{•-} is not an oxidant at physiological pH. It is highly solvent in water and does not cross lipid membranes (83). O₂^{•-} is dismutated to H₂O₂ by superoxide dismutase (SOD). Cardiac myocytes express a cytosolic copper-zinc form of SOD and a mitochondrial manganese form of SOD (MnSOD)(84). The presence of these forms of SOD ensure that O₂^{•-} concentrations are maintained at picomolar concentrations within the cell (85). H₂O₂ is however an excellent candidate for a signalling molecule as it is small and stable and freely crosses membranes to its target cells. It is itself an oxidant but can also react with iron via a Fenton reaction to produce hydroxyl radical, a powerful oxidant. Other candidates for ROS include nitric oxide and peroxynitrite anion (ONOO⁻) formed as a result of reaction between O₂^{•-} and nitric oxide. Cardiac myocytes possess 3 isoforms of nitric oxide synthase (NOS) resulting in the

production of nitric oxide (NO). Types I and III are constitutive and type II is inducible by exposure to cytokines (86, 87). Both NO and ONOO⁻ represent oxidising agents as they can react with target proteins and modify function by oxidation of critical thiol groups.

ROS play an important role in hypoxic responses by modulating cell signaling pathways, and when produced at high levels inducing cell death. ROS interact with cell signaling pathways by way of modification of key thiol groups on proteins that possess regulatory functions. The target proteins may be second messengers such as tyrosine and MAP kinases, tyrosine or serine/threonine protein phosphatases and transcription factors such as NF- κ B (88). ROS also interact with lipids to produce lipid radicals and phospholipases to modulate the activity of arachidonic metabolites that play an integral role in ischemic myocardium (88).

Considerable controversy exists as to whether cellular ROS production increases or decreases during hypoxia. The high reactivity, variable diffusion rate across cell membranes and the instability of ROS make them extremely difficult to detect in cellular systems. Whether cellular ROS increase or decrease during hypoxia it is clear that ROS act as signaling molecules and they are capable of altering cellular redox state and in turn altering protein function. The activity of intracellular second messengers is under the tonic regulation of cellular ROS. For example, epidermal growth factor and insulin-induced intracellular H₂O₂ formation are dependent upon the inhibition of protein tyrosine phosphatase activity by H₂O₂. Tyrosine phosphatase activity is inhibited via reversible oxidation of its catalytic cysteine (89). Under conditions where cellular levels of H₂O₂ are decreased, oxidation of the

catalytic cysteine is reversed and tyrosine phosphatase activity would then increase. Therefore the cellular redox state, being a balance between cellular ROS generation and antioxidant activity determines protein catalytic activity and ultimately functional responses. This is particularly pertinent in the heart where glutathione is highly abundant and is a potent antioxidant when ROS levels are increased such as during reperfusion after ischemia and in the failing heart.

Two major contributors to the production of ROS are the NAD(P)H-oxidase complex and the mitochondria. These two contributors of ROS production are also potential O_2 -sensors.

D. NAD(P)H-oxidase as O_2 -sensor

NAD(P)H-oxidase has been proposed to be a putative O_2 -sensor because the complex is a major source of production of $O_2^{\cdot-}$ in vascular tissue as compared with ROS produced from xanthine oxidase, arachidonic acid and mitochondrial oxidases (90). The complex is a membrane-associated enzyme that reduces O_2 to $O_2^{\cdot-}$ with the transfer of one electron from NADH or NAD(P)H. $O_2^{\cdot-}$ is then converted to H_2O_2 by superoxide dismutase. Therefore H_2O_2 serves to link the O_2 -sensor with the target protein such as an ion channel.

Although the orientation and subunit composition of the enzyme may vary in different tissues, cardiac myocytes appear to express a membrane-bound NAD(P)H-oxidase that is a source of cytosolic $O_2^{\cdot-}$ production (91, 92). The complex is composed of the electron transfer components (gp91phox and

p22phox) and the regulatory p67phox and p47phox subunits. The low molecular weight G protein rac also regulates function of the enzyme.

The oxidase has been postulated to act as a sensor in the regulation of ion channels because the oxidase inhibitor diphenylene iodide (DPI) prevents the inhibition of K⁺ channels by hypoxia (49, 93). The problem with this hypothesis is that DPI also inhibits K⁺ channels (94), and a wide range of flavin-containing oxidases within the cell. In addition, DPI should serve to mimic the hypoxic response. Knockout mice that lack the gp91phox subunit demonstrated a decrease in the generation of ROS but retained the inhibition of K⁺ currents and hypoxic pulmonary vasoconstrictor response (95).

Additional evidence against its role as a sensor comes from patients who suffer from chronic granulomatous disease where one or all of the subunits of the oxidase are absent have normal erythropoietin levels and retain hypoxic vascular responses. Cells derived from these patients are still able to express vascular endothelial growth factor and aldolase mRNA in the presence of 1% O₂ (96). These studies suggest that other isoforms of the oxidase may be involved in O₂-sensing in these patients.

A role for NAD(P)H-oxidase in acute O₂-sensing in cardiac myocytes has not been established. In vascular smooth muscle angiotensin II is a potent activator of NAD(P)H-oxidase and there is good evidence that ROS produced as a result of activation of the oxidase mediates the development of vascular smooth muscle cell hypertrophy, proliferation and migration (97). In cardiac myocytes it has been shown that hypertrophy induced by chronic exposure to glucose (98), α_1 -adrenergic receptor stimulation (99), endothelin-1 or phenylephrine (100, 101) involves signalling by ROS produced from activation

of NAD(P)H-oxidase. Since the rate at which NADP(H)-oxidase produces $O_2^{\cdot-}$ is dependent upon the available substrate (oxygen), hypoxia is associated with less production of $O_2^{\cdot-}$ and a reduced cellular redox state. Reoxygenation results in an increase in production of $O_2^{\cdot-}$ and a shift to an oxidized cellular redox state. NAD(P)H-oxidase has been implicated as being responsible for bursts of ROS associated with reperfusion injury in cardiac myocytes however this remains contentious (102-104). The heart expresses Nox2 and Nox4 isoforms but knockout mice exhibit similar injury after ischemia reperfusion to *wt* mice (105). We tested whether acute activation of NAD(P)H-oxidase with angiotensin II or thrombin could alter cellular superoxide in ventricular myocytes in the presence or absence of hypoxia. Hypoxia (a decrease in oxygen from 150 to 15 mm Hg) decreased superoxide in myocytes assessed as changes in dihydroethidium fluorescence or 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (DCF; Fig. 8A-C)(74, 106). The myocytes possessed functional NAD(P)H-oxidase protein because DPI and apocynin decreased consumption of NADPH (Fig. 8A). However addition of angiotensin II up to 5 μ M concentration could not acutely increase dihydroethidium signal in the myocytes (Fig. 8D and E) despite a robust increase recorded in rat neurons (Fig. 8F). This would suggest that although the protein is functional, NAD(P)H-oxidase does not participate in acute oxygen sensing in guinea pig myocytes. However there is evidence that the oxidase participates in prolonged (chronic) alterations in cellular redox state when the expression of the protein is increased in response to agonists such as angiotensin II and endothelin I during hypertrophic growth (107).

E. Mitochondria as O_2 -sensors

It has been argued that the mitochondria are good candidates for O_2 -sensing since they are capable of rapidly binding and utilising O_2 . In addition, electron transport and oxidative phosphorylation cease in the absence of O_2 . The original basis for the mitochondrial O_2 -sensing hypothesis was that cyanide (which blocks cytochrome oxidase c) stimulated the carotid body (108, 109). It has been suggested that hypoxia induces a decrease in V_{max} for cytochrome oxidase resulting in an increase in $O_2^{\cdot-}$ production due to electron transfer at ubiquinone site (110, 111). Under hypoxic conditions, it is proposed that electron transfer by cytochrome oxidase becomes inhibited and more ubiquinone is generated resulting in an increase in generation of superoxide due to electron transfer to oxygen by ubiquinone. The response is lost in cells **depleted of mitochondrial DNA** (rho(0) cells). However the shift in V_{max} for cytochrome oxidase is dependent upon the available oxygen, the pool of ubiquinone, the reduced state of the electron transfer proteins, the mitochondrial membrane potential and limitations regarding the measurement of $O_2^{\cdot-}$ production (112-114). Increases in ROS production were not evident until 1-2 hours after induction of hypoxia in the cardiomyocytes implying that the duration of hypoxia is also an important consideration (115, 116). Acute exposure to hypoxia (less than hour) does not cause a decrease in V_{max} of cytochrome oxidase. In addition the response of ion channels occurs more rapidly than the rate of inhibition of cytochrome oxidase and a number of ion channels are responsive to levels of hypoxia well above the level that compromises mitochondrial respiration (80 mm Hg vs 2-3 mm Hg). Thiol-specific reducing agents and reduced glutathione mimic the effect of hypoxia on ion channels (74, 75, 79, 81, 117, 118) and consistent with a

decrease in availability of molecular oxygen, a number of groups have also reported that hypoxia results in a decrease in production of ROS (119-123). At least for the renal and pulmonary arteries, it has been argued that the differential responses to hypoxia are due to differential expression of mitochondrial electron transport chain components between cell types (18). It is well recognised that the electron transport chain contributes to the production of superoxide anion by transferring an electron to molecular oxygen. It is on this basis that the electron transport chain is considered to be the oxygen sensor in mitochondria and capable of responding rapidly to acute changes in O_2 tension in cardiac myocytes. When the reduction of oxygen is limited at complex III with application of the complex III inhibitor myxothiazol, or when the electron transport chain is collapsed with application of the cyanide derivative FCCP, superoxide production is decreased (18, 74, 106). This mimics the effect of hypoxia in ventricular myocytes (74, 79, 106). Superoxide anion is very rapidly dismutated to H_2O_2 and H_2O_2 is a stable signalling molecule capable of altering cysteines on target proteins. A decrease in H_2O_2 associated with hypoxia then alters ion channel function either by direct modification of critical thiol groups on the channel protein or by modification of an intermediate protein. This is postulated because dithiothreitol mimics the effect of hypoxia on ion channels (see also Fig. 7) (74, 75, 79, 81, 82, 117, 118) and decreasing intracellular H_2O_2 by perfusing myocytes intracellularly with catalase, (a naturally occurring antioxidant that converts H_2O_2 to water and oxygen) also mimics the effect of hypoxia (79). Therefore mitochondria can rapidly alter cellular redox state in response to

variations in availability of oxygen that then leads to alterations in ion channel function.

III. Implications of hypoxia on the action potential

Variability in delivery of oxygen can lead to electric instability in the myocardium and the generation of arrhythmias (13). A rapid decrease in oxygen supply to cardiac myocytes from 150 to 15 mm Hg is not energy limiting and does not deplete ATP (61) but can alter the function of a number of cardiac ion channels (57, 70, 71, 73-75, 79, 124-126). Under these conditions hypoxia increases late Na^+ current while decreasing fast Na^+ current in rat ventricular myocytes (70, 71, 125). It has been proposed that the increase in late Na^+ current may be arrhythmogenic (127). In addition, acute hypoxia decreases the basal current through L-type Ca^{2+} channels (73-76, 79, 124, 128) and the slow component of the delayed rectifier K^+ channel without affecting the rapid component (57). Application of reduced glutathione or dithiothreitol mimic the effects of hypoxia on late Na^+ current (125), L-type Ca^{2+} channel (74, 75) and delayed rectifier K^+ channel (57) confirming that the ion channels can respond to changes in cellular redox state in response to alterations in oxygen tension. However when the net effects of acute hypoxia (pO_2 of 15-20 mmHg) on Na^+ , Ca^{2+} and K^+ channels are taken into consideration, very little change in action potential duration occurs (Fig. 9A) (129). It is well recognised that ischemic heart disease and angina are associated with an increase in circulating catecholamines that further increases the risk of developing ventricular tachyarrhythmias (130). When the effects of β -adrenergic receptor stimulation on Na^+ , Ca^{2+} and K^+ channels are taken into account, hypoxia causes a prolongation of the action potential that

leads to induction of early afterdepolarizations and spontaneous tachycardia. This can be induced experimentally in ventricular myocytes (Fig. 9B-D) and *in silico* (129). The data are consistent with an increase in sensitivity of the L-type Ca^{2+} channel to isoproterenol when myocytes are exposed to dithiothreitol (74) or perfused intracellularly with catalase (79). This provides clinically relevant insight into the effects of acute hypoxia in the heart during increased adrenergic stimulation. β -Blockers are the only class of antiarrhythmics that can decrease mortality and the mechanism by which they appear to reduce arrhythmias is by decreasing adrenergic stimulation of the L-type Ca^{2+} channel during hypoxia (130). This is consistent with studies that demonstrate that decreasing calcium influx through L-type Ca^{2+} channels or inhibition of protein kinase A (130) or Ca^{2+} /calmodulin-dependent protein kinase II (131-133) can reduce the incidence of early afterdepolarizations.

IV. Concluding remarks

The energy demands of the heart are high. Mitochondria are abundant in the myocardium and are required to meet the energy demands of the heart by supplying ATP during oxidative phosphorylation. In the process they also reduce molecular oxygen to superoxide anion that is rapidly converted to H_2O_2 . The production of reactive species then influences the redox state of the cell and target proteins such as ion channels that possess reactive cysteines. Therefore mitochondria are ideal candidates for acute oxygen sensing in the heart. However, many proteins (including ion channels) can also be modified by nitric oxide and carbon monoxide that can vary during alterations in oxygen tension. It is recognised that there are many mechanisms by which cells respond to acute changes in oxygen tension.

Understanding the mechanisms associated with O₂-sensing and the interaction between sensors and effectors in cardiac myocytes will unravel the pathophysiological processes involved in the induction of arrhythmia. Chronic hypoxia can induce remodelling of the myocardium and development of cardiac hypertrophy and cardiac failure. Under these circumstances alternative oxygen sensing mechanisms may predominate. Understanding oxygen sensing that is specific to the disease process will enable strategies to promote adaptive responses to hypoxia in the myocardium and prevent the deleterious effects associated with O₂ deficits.

Acknowledgments

The research work presented in this article was supported by the National Health and Medical Research Council of Australia (NHMRC) and Australian Research Council (ARC). Livia Hool is an ARC Future Fellow and Honorary NHMRC Senior Research Fellow.

Author Disclosure Statement

There are no disclosures.

References

1. Vagnucci AH, Jr. and Li WW. Alzheimer's disease and angiogenesis. *Lancet* 361: 605-8, 2003.
2. Bazan NG, Palacios-Pelaez R and Lukiw WJ. Hypoxia signaling to genes: significance in Alzheimer's disease. *Mol Neurobiol* 26: 283-98, 2002.
3. Peers C, Dallas ML, Boycott HE, Scragg JL, Pearson HA and Boyle JP. Hypoxia and neurodegeneration. *Ann N Y Acad Sci* 1177: 169-77, 2009.
4. Neuss M, Crow MT, Chesley A and Lakatta EG. Apoptosis in cardiac disease--what is it--how does it occur. *Cardiovasc Drugs Ther* 15: 507-23, 2001.
5. Mari D, Di Berardino F and Cugno M. Chronic heart failure and the immune system. *Clin Rev Allergy Immunol* 23: 325-40, 2002.
6. Semenza GL. Oxygen-regulated transcription factors and their role in pulmonary disease. *Respir Res* 1: 159-62, 2000.
7. Presberg KW and Dincer HE. Pathophysiology of pulmonary hypertension due to lung disease. *Curr Opinion Pul Med* 9: 131-8, 2003.
8. Kemp PJ, Lewis A, Hartness ME, Searle GJ, Miller P, O'Kelly I and Peers C. Airway chemotransduction: from oxygen sensor to cellular effector. *Am J Respir Crit Care Med* 166: S17-S24, 2002.
9. Richard S and French VHL SG. von Hippel-Lindau disease: recent advances and therapeutic perspectives. *Exp Rev Anticancer Ther* 3: 215-33, 2003.
10. Quaegebeur A and Carmeliet P. Oxygen sensing: a common crossroad in cancer and neurodegeneration. *Curr Top Microbiol Immunol* 345: 71-103, 2010.

11. Am Heart Association Heart and Stroke Statistical Update. *Circ* 113: 85-151, 2006.
12. Sanguinetti MC. When the KChIPs are down. *Nature Medicine* 8: 18-9, 2002.
13. Keating MT and Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* 104: 569-80, 2001.
14. Semenza GL and Prabhakar NR. The role of hypoxia-inducible factors in oxygen sensing by the carotid body. *Adv Exp Med Biol* 758: 1-5, 2012.
15. Prabhakar NR. Carbon monoxide (CO) and hydrogen sulfide (H₂S) in hypoxic sensing by the carotid body. *Respir Physiol Neurobiol* 184: 165-9, 2012.
16. Sylvester JT, Shimoda LA, Aaronson PI and Ward JP. Hypoxic pulmonary vasoconstriction. *Physiol Rev* 92: 367-520, 2012.
17. Deshmukh AB, Patel JK, Prajapati AR and Shah S. Perspective in chronic kidney disease: targeting hypoxia-inducible factor (HIF) as potential therapeutic approach. *Ren Fail* 34: 521-32, 2012.
18. Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, Gurtu R and Archer SL. Diversity in mitochondrial function explains differences in vascular oxygen sensing. *Circ Res* 90: 1307-15, 2002.
19. Goldwasser E, LO J, W F and Plzak L. The effect of cobalt on the production of erythropoietin. *Studies Erythropoiesis* 13: 55-60, 1958.
20. Wang GL, Jiang BH, Rue EA and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proceedings of the Nat Acad Sci USA* 92: 5510-4, 1995.

21. Epstein A, Gleadle J, McNeill L, Hewitson K, O'Rourke J, Mole D, Mukherji M, Metzen E, Wilson M, Dhanda A, Tian Y-M, Masson M, Hamilton D, Jaakkola P, Barstead R, Hodgkin J, Maxwell P, Pugh C, Schofield C and Ratcliffe P. *C.elegans* EGL-9 and Mammalian Homologs Define a Family of Dioxygenases that Regulate HIF by Prolyl Hydroxylation. *Cell* 107: 43-54, 2001.
22. Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, Gassmann M and Candinas D. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *FASEB J* 15: 2445-53, 2001.
23. Breitingner HG, Villmann C, Rennert J, Ballhausen D and Becker CM. Hydroxylated residues influence desensitization behaviour of recombinant alpha3 glycine receptor channels. *J Neurochem* 83: 30-6, 2002.
24. Villarroel A, Herlitze S, Witzemann V, Koenen M and Sakmann B. Asymmetry of the rat acetylcholine receptor subunits in the narrow region of the pore. *Proceedings of the Royal Soc Lon - Series B: Biol Sci* 249: 317-24, 1992.
25. Takahashi N, Kuwaki T, Kiyonaka S, Numata T, Kozai D, Mizuno Y, Yamamoto S, Naito S, Knevels E, Carmeliet P, Oga T, Kaneko S, Suga S, Nokami T, Yoshida J and Mori Y. TRPA1 underlies a sensing mechanism for O₂. *Nat Chem Biol* 7: 701-11, 2011.
26. Pozsgai G, Bodkin JV, Graepel R, Bevan S, Andersson DA and Brain SD. Evidence for the pathophysiological relevance of TRPA1 receptors in the cardiovascular system in vivo. *Cardiovasc Res* 87: 760-8, 2010.

27. Lopez-Lopez JR and Gonzalez C. Time course of K⁺ current inhibition by low oxygen in chemoreceptor cells of adult rabbit carotid body. Effects of carbon monoxide. *FEBS Letters* 299: 251-4, 1992.
28. Riesco-Fagundo A, Perez-Garcia M, Gonzalez C and Lopez-Lopez JR. O₂ modulates maxiK channels of rat chemoreceptor cells by a membrane restricted and CO sensitive mechanism. *Circ Res* 89: 430-6, 2001.
29. Tamayo L, Lopez-Lopez JR, Castaneda J and Gonzalez C. Carbon monoxide inhibits hypoxic pulmonary vasoconstriction in rats by a cGMP-independent mechanism. *Pflug Archiv - Eur J Physiol* 434: 698-704, 1997.
30. Williams SEJ, Wootton P, Mason HS, Bould J, Iles DE, Riccardi D, Peers C and Kemp PJ. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. *Science* 306: 2093-7, 2004.
31. Han F, Takeda K, Ono M, Date F, Ishikawa K, Yokoyama S, Shinozawa Y, Furuyama K and Shibahara S. Hypoxemia induces expression of heme oxygenase-1 and heme oxygenase-2 proteins in the mouse myocardium. *J Biochem* 147: 143-51, 2010.
32. Yet SF, Tian R, Layne MD, Wang ZY, Maemura K, Solovyeva M, Ith B, Melo LG, Zhang L, Ingwall JS, Dzau VJ, Lee ME and Perrella MA. Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. *Circ Res* 89: 168-73, 2001.
33. Yoshida T, Maulik N, Ho YS, Alam J and Das DK. H(mox-1) constitutes an adaptive response to effect antioxidant cardioprotection: A study with transgenic mice heterozygous for targeted disruption of the Heme oxygenase-1 gene. *Circ* 103: 1695-701, 2001.

34. Ding B, Gibbs PE, Brookes PS and Maines MD. The coordinated increased expression of biliverdin reductase and heme oxygenase-2 promotes cardiomyocyte survival: a reductase-based peptide counters beta-adrenergic receptor ligand-mediated cardiac dysfunction. *Faseb J* 25: 301-13, 2011.
35. Rosa AO, Movafagh S, Cleemann L and Morad M. Hypoxic regulation of cardiac Ca²⁺ channel: possible role of haem oxygenase. *J Physiol* 590: 4223-37, 2012.
36. Grochot-Przeczek A, Dulak J and Jozkowicz A. Haem oxygenase-1: non-canonical roles in physiology and pathology. *Clin Sci (Lond)* 122: 93-103, 2012.
37. Wilkinson WJ and Kemp PJ. Carbon monoxide: an emerging regulator of ion channels. *J Physiol* 589: 3055-62, 2011.
38. Dallas ML, Boyle JP, Milligan CJ, Sayer R, Kerrigan TL, McKinstry C, Lu P, Mankouri J, Harris M, Scragg JL, Pearson HA and Peers C. Carbon monoxide protects against oxidant-induced apoptosis via inhibition of Kv2.1. *Faseb J* 25: 1519-30, 2011.
39. Scragg JL, Dallas ML, Wilkinson JA, Varadi G and Peers C. Carbon monoxide inhibits L-type Ca²⁺ channels via redox modulation of key cysteine residues by mitochondrial reactive oxygen species. *J Biol Chem* 283: 24412-9, 2008.
40. Dallas ML, Yang Z, Boyle JP, Boycott HE, Scragg JL, Milligan CJ, Elies J, Duke A, Thireau J, Reboul C, Richard S, Bernus O, Steele DS and Peers C. Carbon monoxide induces cardiac arrhythmia via induction of the late Na⁺ current. *Am J Respir Crit Care Med* 186: 648-56, 2012.

41. Peers C and Steele DS. Carbon monoxide: a vital signalling molecule and potent toxin in the myocardium. *J Mol Cell Cardiol* 52: 359-65, 2012.
42. Yi L, Morgan JT and Ragsdale SW. Identification of a thiol/disulfide redox switch in the human BK channel that controls its affinity for heme and CO. *J Biol Chem* 285: 20117-27, 2010.
43. Telezhkin V, Brazier SP, Mears R, Muller CT, Riccardi D and Kemp PJ. Cysteine residue 911 in C-terminal tail of human BK(Ca) α channel subunit is crucial for its activation by carbon monoxide. *Pflugers Arch* 461: 665-75, 2011.
44. Yusifov T, Savalli N, Gandhi CS, Ottolia M and Olcese R. The RCK2 domain of the human BKCa channel is a calcium sensor. *Proc Natl Acad Sci U S A* 105: 376-81, 2008.
45. Foresti R, Hammad J, Clark JE, Johnson TR, Mann BE, Friebe A, Green CJ and Motterlini R. Vasoactive properties of CORM-3, a novel water-soluble carbon monoxide-releasing molecule. *Br J Pharmacol* 142: 453-60, 2004.
46. Musameh MD, Fuller BJ, Mann BE, Green CJ and Motterlini R. Positive inotropic effects of carbon monoxide-releasing molecules (CO-RMs) in the isolated perfused rat heart. *Br J Pharmacol* 149: 1104-12, 2006.
47. Lopez-Barneo J, Lopez-Lopez JR, Urena J and Gonzalez C. Chemotransduction in the carotid body: K⁺ current modulated by PO₂ in type I chemoreceptor cells. *Science* 241: 580-2, 1988.
48. O'Kelly I, Stephens RH, Peers C and Kemp PJ. Potential identification of the O₂-sensitive K⁺ current in a human neuroepithelial body-derived cell line. *Am J Physiol* 276: L96-L104, 1999.

49. Youngson C, Nurse C, Yeger H and Cutz E. Oxygen sensing in airway chemoreceptors. *Nature* 365: 153-5, 1993.
50. Delpiano MA and Hescheler J. Evidence for a PO₂-sensitive K⁺ channel in the type-I cell of the rabbit carotid body. *FEBS Letters* 249: 195-8, 1989.
51. Thompson RJ, Jackson A and Nurse CA. Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells. *J Physiol* 498: 503-10, 1997.
52. Post JM, Hume JR, Archer SL and Weir EK. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. *Am J Physiol* 262: C882-C90, 1992.
53. Franco-Obregon A and Lopez-Barneo J. Differential oxygen sensitivity of calcium channels in rabbit smooth muscle cells of conduit and resistance pulmonary arteries. *J Physiol* 491: 511-8, 1996.
54. Urena J, Franco-Obregon A and Lopez-Barneo J. Contrasting effects of hypoxia on cytosolic Ca²⁺ spikes in conduit and resistance myocytes of the rabbit pulmonary artery. *J Physiol* 496: 103-9, 1996.
55. Bakhramov A, Evans AM and Kozlowski RZ. Differential effects of hypoxia on the intracellular Ca²⁺ concentration of myocytes isolated from different regions of the rat pulmonary arterial tree. *Exper Physiol* 83: 337-47, 1998.
56. Shimoda LA and Polak J. Hypoxia. 4. Hypoxia and ion channel function. *Am J Physiol Cell Physiol* 300: C951-67, 2011.
57. Hool LC. Differential regulation of the slow and rapid components of guinea-pig cardiac delayed rectifier K⁺ channels by hypoxia. *J Physiol* 554: 743-54, 2004.

58. Overholt JL, Ficker E, Yang T, Shams H, Bright GR and Prabhakar NR. Chemosensing at the carotid body. Involvement of a HERG-like potassium current in glomus cells. *Advan Exper Med Biol* 475: 241-8, 2000.
59. Franco-Obregon A, Urena J and Lopez-Barneo J. Oxygen-sensitive calcium channels in vascular smooth muscle and their possible role in hypoxic arterial relaxation. *Proc Nat Acad Sci USA* 92: 4715-9, 1995.
60. Franco-Obregon A and Lopez-Barneo J. Low PO₂ inhibits calcium channel activity in arterial smooth muscle cells. *Am J Physiol* 271: H2290-H9, 1996.
61. Casey TM, Pakay JL, Guppy M and Arthur PG. Hypoxia causes downregulation of protein and RNA synthesis in noncontracting Mammalian cardiomyocytes. *Circ Res* 90: 777-83, 2002.
62. Stumpe T and Schrader J. Phosphorylation potential, adenosine formation, and critical PO₂ in stimulated rat cardiomyocytes. *Am J Physiol* 273: H756-H66, 1997.
63. Noma A. ATP-regulated K⁺ channels in cardiac muscle. *Nature* 305: 147-8, 1983.
64. Seino S and Miki T. Gene targeting approach to clarification of ion channel function: studies of Kir6.x null mice. *J Physiol* 554: 295-300, 2004.
65. Sato T, Sasaki N, Seharaseyon J, O'Rourke B and Marban E. Selective pharmacological agents implicate mitochondrial but not sarcolemmal K(ATP) channels in ischemic cardioprotection. *Circ* 101: 2418-23, 2000.
66. Wang Y, Hirai K and Ashraf M. Activation of mitochondrial ATP-sensitive K(+) channel for cardiac protection against ischemic injury is dependent on protein kinase C activity. *Cir Res* 85: 731-41, 1999.

67. Light PE, Sabir AA, Allen BG, Walsh MP and French RJ. Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K⁺ channels. A possible mechanistic link to ischemic preconditioning. *Circ Res* 79: 399-406, 1996.
68. Ohnuma Y, Miura T, Miki T, Tanno M, Kuno A, Tsuchida A and Shimamoto K. Opening of mitochondrial K(ATP) channel occurs downstream of PKC-epsilon activation in the mechanism of preconditioning. *Am J Physiol* 283: H440-H7, 2002.
69. Han J, Kim E, Ho WK and Earm YE. Sulfhydryl redox modulates ATP-sensitive K⁺ channels in rabbit ventricular myocytes. *Biochem Biophys Res Comm* 219: 900-3, 1996.
70. Ju YK, Saint DA and Gage PW. Inactivation-resistant channels underlying the persistent sodium current in rat ventricular myocytes. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 256: 163-8, 1994.
71. Ju YK, Saint DA and Gage PW. Hypoxia increases persistent sodium current in rat ventricular myocytes. *J Physiol* 497: 337-47, 1996.
72. Hammarstrom AK and Gage PW. Hypoxia and persistent sodium current. *Eur Biophys J* 31: 323-30, 2002.
73. Fearon IM, Palmer AC, Balmforth AJ, Ball SG, Mikala G, Schwartz A and Peers C. Hypoxia inhibits the recombinant alpha 1C subunit of the human cardiac L-type Ca²⁺ channel. *J Physiol* 500: 551-6, 1997.
74. Hool LC. Hypoxia increases the sensitivity of the L-type Ca(2+) current to beta-adrenergic receptor stimulation via a C2 region-containing protein kinase C isoform. *Circ Res* 87: 1164-71, 2000.

75. Hool LC. Hypoxia alters the sensitivity of the L-type Ca^{2+} channel to alpha-adrenergic receptor stimulation in the presence of beta-adrenergic receptor stimulation. *Circ Res* 88: 1036-43, 2001.
76. Fearon IM, Varadi G, Koch SE, Isaacsohn I, Ball SG and Peers C. Splice variants reveal the region involved in oxygen sensing by recombinant human L-type Ca^{2+} channels. *Circ Res* 87: 537-9, 2000.
77. Lebuffe G, Schumacker PT, Shao ZH, Anderson T, Iwase H and vanden Hoek TL. ROS and NO trigger early preconditioning: relationship to mitochondrial KATP channel. *Am J Physiol - Heart & Circulatory Physiology* 284: H299-H308, 2003.
78. Liu Y and Gutterman DD. Oxidative stress and potassium channel function. *Clinical & Experimental Pharmacology & Physiology* 29: 305-11, 2002.
79. Hool LC and Arthur PG. Decreasing cellular hydrogen peroxide with catalase mimics the effects of hypoxia on the sensitivity of the L-type Ca^{2+} channel to beta-adrenergic receptor stimulation in cardiac myocytes. *Circ Res* 91: 601-9, 2002.
80. Anzai K, Ogawa K, Ozawa T and Yamamoto H. Oxidative modification of ion channel activity of ryanodine receptor. *Antioxidants & Redox Signaling* 2: 35-40, 2000.
81. Campbell DL, Stamler JS and Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. *Journal of General Physiology* 108: 277-93, 1996.

82. Tang H, Viola HM, Filipovska A and Hool LC. Ca(v)1.2 calcium channel is glutathionylated during oxidative stress in guinea pig and ischemic human heart. *Free Radic Biol Med* 51: 1501-11, 2011.
83. Khan AU and Wilson T. Reactive oxygen species as cellular messengers. *Chemistry & Biology* 2: 437-45, 1995.
84. Cepinskas G, Rui T and Kvietys PR. Interaction between reactive oxygen metabolites and nitric oxide in oxidant tolerance. *Free Radical Biology & Medicine* 33: 433-40, 2002.
85. Stone JR and Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* 8: 243-70, 2006.
86. Balligand JL, Kobzik L, Han X, Kaye DM, Belhassen L, O'Hara DS, Kelly RA, Smith TW and Michel T. Nitric oxide-dependent parasympathetic signaling is due to activation of constitutive endothelial (type III) nitric oxide synthase in cardiac myocytes. *Journal of Biological Chemistry* 270: 14582-6, 1995.
87. Balligand JL, Ungureanu-Longrois D, Simmons WW, Pimental D, Malinski TA, Kapturczak M, Taha Z, Lowenstein CJ, Davidoff AJ and Kelly RA. Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes. Characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes in vitro. *J Biol Chem* 269: 27580-8, 1994.
88. Wolin MS. Interactions of oxidants with vascular signaling systems. *Arter Thromb Vasc Biol* 20: 1430-42, 2000.

89. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK and Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* 423: 769-73, 2003.
90. Griendling KK, Sorescu D and Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 86: 494-501, 2000.
91. Xiao L, Pimentel DR, Wang J, Singh K, Colucci WS and Sawyer DB. Role of reactive oxygen species and NAD(P)H oxidase in alpha(1)-adrenoceptor signaling in adult rat cardiac myocytes. *Am J Physiol* 282: C926-C34, 2002.
92. Mohazzab H, Kaminski PM and Wolin MS. Lactate and PO₂ modulate superoxide anion production in bovine cardiac myocytes: potential role of NADH oxidase. *Circ* 96: 614-20, 1997.
93. O'Kelly I, Peers C and Kemp PJ. NADPH oxidase does not account fully for O₂-sensing in model airway chemoreceptor cells. *Biochem Biophys Res Comm* 283: 1131-4, 2001.
94. Weir EK, Wyatt CN, Reeve HL, Huang J, Archer SL and Peers C. Diphenyleneiodonium inhibits both potassium and calcium currents in isolated pulmonary artery smooth muscle cells. *J Appl Physiol* 76: 2611-5, 1994.
95. Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, Dinauer MC and Weir EK. O₂ sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. *Proc Nat Acad Sci USA* 96: 7944-9, 1999.
96. Wenger RH, Marti HH, Schuerer-Maly CC, Kvietikova I, Bauer C, Gassmann M and Maly FE. Hypoxic induction of gene expression in chronic granulomatous disease-derived B-cell lines: oxygen sensing is independent of the cytochrome b558-containing nicotinamide adenine dinucleotide phosphate oxidase. *Blood* 87: 756-61, 1996.

97. Lee MY and Griending KK. Redox signaling, vascular function, and hypertension. *Antioxid Redox Signal* 10: 1045-59, 2008.
98. Privratsky JR, Wold LE, Sowers JR, Quinn MT and Ren J. AT1 blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: role of the AT1 receptor and NADPH oxidase. *Hypertens* 42: 206-12, 2003.
99. Xiao L, Pimentel DR, Wang J, Singh K, Colucci WS and Sawyer DB. Role of reactive oxygen species and NAD(P)H oxidase in alpha(1)-adrenoceptor signaling in adult rat cardiac myocytes. *Am J Physiol - Cell Physiology* 282: C926-C34, 2002.
100. Tanaka K, Honda M and Takabatake T. Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. *Journal of the Am College of Cardiology* 37: 676-85, 2001.
101. Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, Yamaguchi O, Mano T, Matsumura Y, Ueno H, Tada M and Hori M. Involvement of nuclear factor-kappaB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy. *Circ* 105: 509-15, 2002.
102. Hoffmeyer MR, Jones SP, Ross CR, Sharp B, Grisham MB, Laroux FS, Stalker TJ, Scalia R and Lefer DJ. Myocardial ischemia/reperfusion injury in NADPH oxidase-deficient mice. *Circ Res* 87: 812-7, 2000.
103. Oudot A, Vergely C, Ecarnot-Laubriet A and Rochette L. Angiotensin II activates NADPH oxidase in isolated rat hearts subjected to ischaemia-reperfusion. *Eur J Pharmacol* 462: 145-54, 2003.
104. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A and Ruggiero F. Decrease in mitochondrial complex I activity in

- ischemic/reperfused rat heart. Involvement of reactive oxygen species and cardiolipin. *Circ Res* 94: 53-9, 2004.
105. Matsushima S, Kuroda J, Ago T, Zhai P, Ikeda Y, Oka S, Fong GH, Tian R and Sadoshima J. Broad suppression of NADPH oxidase activity exacerbates ischemia/reperfusion injury through inadvertent downregulation of hypoxia-inducible factor-1alpha and upregulation of peroxisome proliferator-activated receptor-alpha. *Circ Res* 112: 1135-49, 2013.
106. Hool LC, Di Maria CA, Viola HM and Arthur PG. Role of NAD(P)H oxidase in the regulation of cardiac L-type Ca²⁺ channel function during acute hypoxia. *Cardiovasc Res* 67: 624-35, 2005.
107. Laplante MA and de Champlain J. The interrelation of the angiotensin and endothelin systems on the modulation of NAD(P)H oxidase. *Can J Physiol Pharmacol* 84: 21-8, 2006.
108. Mulligan E, Lahiri S and Storey BT. Carotid body O₂ chemoreception and mitochondrial oxidative phosphorylation. *J App Physiol: Respir, Environ Ex Physiol* 51: 438-46, 1981.
109. Biscoe TJ and Duchon MR. Cellular basis of transduction in carotid chemoreceptors. *Am J Physiol* 258: L271-L8, 1990.
110. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM and Schumacker PT. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O₂ sensing. *J Biol Chem* 275: 25130-8, 2000.
111. Waypa GB, Chandel NS and Schumacker PT. Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. *Circ Res* 88: 1259-66, 2001.

112. Korshunov SS, Skulachev VP and Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 416: 15-8, 1997.
113. Turrens JF and Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 191: 421-7, 1980.
114. Swift LM and Sarvazyan N. Localization of dichlorofluorescein in cardiac myocytes: implications for assessment of oxidative stress. *Am J Physiol - Heart & Circulatory Physiology* 278: H982-H990, 2000.
115. vanden Hoek TL, Becker LB, Shao Z, Li C and Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 273: 18092-8, 1998.
116. Becker LB, vanden Hoek TL, Shao ZH, Li CQ and Schumacker PT. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *Am J Physiol* 277: H2240-H2246, 1999.
117. Park MK, Lee SH, Ho WK and Earm YE. Redox agents as a link between hypoxia and the responses of ionic channels in rabbit pulmonary vascular smooth muscle. *Exp Physiol* 80: 835-42, 1995.
118. Park MK, Lee SH, Lee SJ, Ho WK and Earm YE. Different modulation of Ca-activated K channels by the intracellular redox potential in pulmonary and ear arterial smooth muscle cells of the rabbit. *Pflug Archiv - Eur J Physiol* 430: 308-14, 1995.
119. Weir EK and Archer SL. The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. *FASEB J* 9: 183-9, 1995.
120. Michelakis ED, Rebeyka I, Wu X, Nsair A, Thebaud B, Hashimoto K, Dyck JR, Haromy A, Harry G, Barr A and Archer SL. O₂ sensing in the human

ductus arteriosus: regulation of voltage-gated K⁺ channels in smooth muscle cells by a mitochondrial redox sensor. *Circ Res* 91: 478-86, 2002.

121. Kroll SL and Czyzyk-Krzeska MF. Role of H₂O₂ and heme-containing O₂ sensors in hypoxic regulation of tyrosine hydroxylase gene expression. *Am J Physiol* 274: C167-C74, 1998.

122. Weir EK, Hong Z, Porter VA and Reeve HL. Redox signaling in oxygen sensing by vessels. *Respir Physiol Neurobiol* 132: 121-30, 2002.

123. Hanson ES and Leibold EA. Regulation of iron regulatory protein 1 during hypoxia and hypoxia/reoxygenation. *J Biol Chem* 273: 7588-93, 1998.

124. Smani T, Hernandez A, Urena J, Castellano AG, Franco-Obregon A, Ordonez A and Lopez-Barneo J. Reduction of Ca(2⁺) channel activity by hypoxia in human and porcine coronary myocytes. *Cardiovasc Res* 53: 97-104, 2002.

125. Wang W, Ma J, Zhang P and Luo A. Redox reaction modulates transient and persistent sodium current during hypoxia in guinea pig ventricular myocytes. *Pflugers Arch* 454: 461-75, 2007.

126. Hool LC. Acute hypoxia differentially regulates K(+) channels. Implications with respect to cardiac arrhythmia. *Eur Biophys J* 34: 369-76, 2005.

127. Saint DA. The cardiac persistent sodium current: an appealing therapeutic target? *Br J Pharmacol* 153: 1133-42, 2008.

128. Fearon IM, Palmer AC, Balmforth AJ, Ball SG, Varadi G and Peers C. Modulation of recombinant human cardiac L-type Ca²⁺ channel alpha1C subunits by redox agents and hypoxia. *J Physiol* 514: 629-37, 1999.

129. Gaur N, Rudy Y and Hool L. Contributions of ion channel currents to ventricular action potential changes and induction of early afterdepolarizations during acute hypoxia. *Circ Res* 105: 1196-203, 2009.
130. Dorian P. Antiarrhythmic action of beta-blockers: potential mechanisms. *J Cardiovasc Pharmacol Ther* 10 Suppl 1: S15-22, 2005.
131. Mazur A, Roden DM and Anderson ME. Systemic administration of calmodulin antagonist W-7 or protein kinase A inhibitor H-8 prevents torsade de pointes in rabbits. *Circ* 100: 2437-42, 1999.
132. Thomas G, Killeen MJ, Grace AA and Huang CL. Pharmacological separation of early afterdepolarizations from arrhythmogenic substrate in DeltaKPQ Scn5a murine hearts modelling human long QT 3 syndrome. *Acta Physiol (Oxf)* 192: 505-17, 2008.
133. Gallacher DJ, Van de Water A, van der Linde H, Hermans AN, Lu HR, Towart R and Volders PG. In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome. *Cardiovasc Res* 76: 247-56, 2007.

Figure Legends

Figure 1. Proposed mechanisms of action of heme oxygenase-1 and heme oxygenase-2 in ventricular myocytes. Heme oxygenase can directly bind to the Ca^{2+} /calmodulin-dependent protein kinase II-specific motif on the C terminal end of α subunit of the L-type Ca^{2+} channel ($\text{Ca}_v1.2$). Alternatively heme oxygenase is converted to ferrous iron (Fe^{2+}), carbon monoxide and biliverdin. Of the three products carbon monoxide regulates signalling through alterations in production of reactive oxygen species (ROS) from the mitochondria and nitric oxide (NO) production via soluble guanylate cyclase (sGC) or nitric oxide synthase (NOS). Carbon monoxide also influences MAPK and protein kinase A (PKA) activity. For further detail please see text.

Figure 2. Purified human long NT isoform of $\text{Ca}_v1.2$ channel reconstituted in liposomes can be assessed using single channel patch-clamp technique. (A) Representative single-channel currents recorded at +100 mV. The channel is open in an upward direction. 'C' denotes the channels' closed state and 'O' the open state of the respective channel (n) contributing to the total current obtained in the recording. A current-amplitude frequency histogram constructed from 20 s of single-channel data recorded at +100 mV is shown below. (B) Representative single-channel currents recorded at varying pipette potentials as indicated. Nisoldipine (niso) abolished channel activity in the patch. External and pipette solutions contained 100 mM BaCl_2 , 50 mM NaCl, 10 mM Hepes, 2 μM BayK8644, pH 7.4 at 22 °C. (C) I–V relationship recorded from the same patch in the absence and presence of nisoldipine. (D) Immunoblot of the long NT isoform

of the Ca_v1.2 channel protein indicated at approximately 240 kDa when probed with anti-Ca_v1.2 antibody (lane 1) and protein from HEK293 cells expressing pcDNA3.1 plasmid minus α1C,77L-His (lane 2). Reproduced with permission from Ref. 82.

Figure 3. The carbon monoxide donor CORM2 does not directly alter the open probability (P_o) of the purified human long NT isoform of Ca_v1.2 reconstituted in liposomes. (A) Representative single-channel currents recorded at +100 mV in control Ca_v1.2 protein (upper trace) and in Ca_v1.2 channel protein exposed to 30 μM CORM2 (middle trace). The L-type calcium channel antagonist nisoldipine (Nisol) attenuated the openings (lower trace). External and pipette solutions contained 100 mM BaCl₂, 50 mM NaCl, 10 mM Hepes, 2 μM BayK8644, pH 7.4 at 22 °C. (B) Mean ±SE of alterations in P_o relative to P_o recorded in control Ca_v1.2 (at left) and I–V relationship recorded from Ca_v1.2 channels in the absence and presence of CORM2.

Figure 4. Hypoxia does not directly alter the open probability (P_o) of the purified human long NT isoform of Ca_v1.2 or the short NT isoform of Ca_v1.2 reconstituted in liposomes. (A) Representative single-channel currents recorded at +100 mV in control long NT isoform of Ca_v1.2 protein in room oxygen (upper trace) and during exposure to hypoxia (pO₂ 15 mmHg) (lower trace). External and pipette solutions contained 100 mM BaCl₂, 50 mM NaCl, 10 mM Hepes, 2 μM BayK8644, pH 7.4 at 22 °C. (B) Mean ±SE of alterations in P_o during hypoxia relative to P_o recorded in control long NT isoform and short NT isoform as indicated (at left) and I–V relationship

recorded from long NT isoform of $\text{Ca}_v1.2$ channels in the absence and presence of hypoxia.

Figure 5. Exposure of guinea-pig ventricular myocytes to hypoxia induces a rapid decrease in basal macroscopic L-type Ca^{2+} current. (A) Time course of changes in membrane current recorded during exposure to hypoxic Tyrode's solution (pO_2 of 17 mm Hg). a, b, and c refer to time points at which membrane currents (shown in B) were recorded. (B) Membrane currents recorded at time points in protocol illustrated in panel A. (C) Mean $\pm$ SE of current-voltage (*I-V*) relationships measured in 9 cells during voltage steps from -60 mV to +80 mV. *I-V*s were recorded in 2 mM nisoldipine (nisol) to indicate that calcium currents recorded were L-type. Reproduced with permission from Ref. 74.

Figure 6. Schematic representing positions of cysteines on the long NT isoform of $\text{Ca}_v1.2$ channel protein. Cysteines are shown as black dots. $\text{Ca}_v1.2$ protein (α subunit) is comprised of 4 heterotetramers (I–IV) each with 6 transmembrane spanning regions (S1–S6). P= ion conducting pore region. Voltage sensing region S4 is denoted with +++

Figure 7. Modification of thiol groups on the human long NT isoform of $\text{Ca}_v1.2$ alters function. (A) Representative single-channel currents recorded at -100 mV in the absence and presence of 200 μM DTNB followed by 1 mM DTT and then 2 μM nisoldipine, including the P_o for each treatment as shown. (B) Means $\pm$ SE of channel open probability for all patches exposed to DTNB,

DTT, and nisoldipine (Nisol) as indicated and I–V relationship for currents from experiment (A). *P<0.05. Reproduced with permission from Ref. 82.

Figure 8. Adult guinea pig cardiac myocytes express functional NAD(P)H oxidase but cannot increase superoxide in response to stimulation by angiotensin II. (A) NADPH consumption in the presence of 100 μ M DPI and 500 μ M apocynin (Apo) assessed by spectrophotometry from 5 hearts. (B). Hypoxia is associated with a decrease in cellular superoxide. Dihydroethidium (DHE) fluorescence recorded from a cell exposed to normoxia only and in another cell exposed to normoxia followed by hypoxia (Normoxia/hypoxia); a.u.=arbitrary units. The superoxide scavenger N-tert-butyl-alpha-phenyl-nitrone (PBN) caused 90 $\pm$ 3% decrease in the rate of DHE signal (inset right). (C) Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by hypoxia (Normoxia/hypoxia). (D) DHE fluorescence recorded from a cell exposed to hypoxia only and in another cell exposed to hypoxia followed by 5 μ M angiotensin II (Ang II) (Hypoxia/Ang II). Ratio of fluorescence for cells exposed to hypoxia only and for cells exposed to hypoxia followed by Ang II (Hypoxia/Ang II) is shown inset right. (E). Ang II does not alter superoxide during exposure to room oxygen in cardiac myocytes. DHE fluorescence recorded from a cell exposed to normoxia only and in another cell exposed to normoxia followed by addition of 5 μ M Ang II at 30 min (Normoxia/Ang II). Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by Ang II (Normoxia/Ang II) is shown inset right. (F) Ang II increases cellular superoxide in embryonic cortical neurons. DHE

fluorescence recorded from a neuron exposed to normoxia only and in another neuron exposed to normoxia followed by 5 μ M Ang II (Normoxia/Ang II). Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by Ang II (Normoxia/Ang II) is shown inset at right. Reproduced with permission from Ref. 106.

Figure 9. Representative experimental traces demonstrating the effects of hypoxia and β -adrenergic receptor stimulation on action potentials (AP) paced at 1000-ms cycle length in a guinea pig ventricular myocyte.

(A) Effect of reducing pO_2 from 150 mm Hg (normoxia) to 17 mm Hg (hypoxia) on the AP. (B) Effect of hypoxia + 3 nM isoproterenol (β -adrenergic receptor agonist) on the AP. (C) Progression to early afterdepolarization formation in the same cell. (D) Progression to sustained activity in the same cell. In D, the myocyte was not stimulated and APs were spontaneous. See text for further detail. Reproduced with permission from Ref.129.

Figure 1

Figure 1. Proposed mechanisms of action of heme oxygenase-1 and heme oxygenase-2 in ventricular myocytes. Heme oxygenase can directly bind to the Ca²⁺/calmodulin-dependent protein kinase II-specific motif on the C terminal end of α subunit of the L-type Ca²⁺ channel (Cav1.2). Alternatively heme oxygenase is converted to ferrous iron (Fe²⁺), carbon monoxide and biliverdin. Of the three products carbon monoxide regulates signalling through alterations in production of reactive oxygen species (ROS) from the mitochondria and nitric oxide (NO) production via soluble guanylate cyclase (sGC) or nitric oxide synthase (NOS). Carbon monoxide also influences MAPK and protein kinase A (PKA) activity. For further detail please see text.

Figure 2

Figure 2. Purified human long NT isoform of $\text{Ca}_v1.2$ channel reconstituted in liposomes can be assessed using single channel patch-clamp technique. (A) Representative single-channel currents recorded at +100 mV. The channel is open in an upward direction. 'C' denotes the channels' closed state and 'O' the open state of the respective channel (n) contributing to the total current obtained in the recording. A current-amplitude frequency histogram constructed from 20 s of single-channel data recorded at +100 mV is shown below. (B) Representative single-channel currents recorded at varying pipette potentials as indicated. Nisoldipine (nisol) abolished channel activity in the patch. External and pipette solutions contained 100 mM BaCl_2 , 50 mM NaCl, 10 mM Hepes, 2 μM BayK8644, pH 7.4 at 22 °C. (C) I–V relationship recorded from the same patch in the absence and presence of nisoldipine. (D) Immunoblot of the long NT isoform of the $\text{Ca}_v1.2$ channel protein indicated at approximately 240 kDa when probed with anti- $\text{Ca}_v1.2$ antibody (lane 1) and protein from HEK293 cells expressing pcDNA3.1 plasmid minus $\alpha 1\text{C}, 77\text{L-His}$ (lane 2). Reproduced with permission from Ref. 82.

Figure 3

Figure 3. The carbon monoxide donor CORM2 does not directly alter the open probability (P_o) of the purified human long NT isoform of $Ca_v1.2$ reconstituted in liposomes. (A) Representative single-channel currents recorded at +100 mV in control $Ca_v1.2$ protein (upper trace) and in $Ca_v1.2$ channel protein exposed to 30 μ M CORM2 (middle trace). The L-type calcium channel antagonist nisoldipine (Nisol) attenuated the openings (lower trace). External and pipette solutions contained 100 mM $BaCl_2$, 50 mM NaCl, 10 mM Hepes, 2 μ M BayK8644, pH 7.4 at 22 $^{\circ}$ C. (B) Mean $\pm$ SE of alterations in P_o relative to P_o recorded in control $Ca_v1.2$ (at left) and I–V relationship recorded from $Ca_v1.2$ channels in the absence and presence of CORM2.

Figure 4

Figure 4. Hypoxia does not directly alter the open probability (P_o) of the purified human long NT isoform of $Ca_v1.2$ or the short NT isoform of $Ca_v1.2$ reconstituted in liposomes. (A) Representative single-channel currents recorded at +100 mV in control long NT isoform of $Ca_v1.2$ protein in room oxygen (upper trace) and during exposure to hypoxia (pO_2 15 mmHg) (lower trace)(74). External and pipette solutions contained 100 mM $BaCl_2$, 50 mM NaCl, 10 mM Hepes, 2 μ M BayK8644, pH 7.4 at 22 °C. (B) Mean $\pm$ SE of alterations in P_o during hypoxia relative to P_o recorded in control long NT isoform and short NT isoform as indicated (at left) and I-V relationship recorded from long NT isoform of $Ca_v1.2$ channels in the absence and presence of hypoxia.

Figure 5

Figure 5. Exposure of guinea-pig ventricular myocytes to hypoxia induces a rapid decrease in basal macroscopic L-type Ca^{2+} current. (A) Time course of changes in membrane current recorded during exposure to hypoxic Tyrode's solution (pO_2 of 17 mm Hg). a, b, and c refer to time points at which membrane currents (shown in B) were recorded. (B) Membrane currents recorded at time points in protocol illustrated in panel A. (C) Mean $\pm$ SE of current-voltage (I - V) relationships measured in 9 cells during voltage steps from -60 mV to +80 mV. I - V s were recorded in 2 mM nisoldipine (nisol) to indicate that calcium currents recorded were L-type. Reproduced with permission from Ref. 74.

Figure 6

Figure 6. Schematic representing positions of cysteines on the long NT isoform of $\text{Ca}_v1.2$ channel protein. Cysteines are shown as black dots.

$\text{Ca}_v1.2$ protein (α subunit) is comprised of 4 heterotetramers (I –IV) each with 6 transmembrane spanning regions (S1-S6). P= ion conducting pore region.

Voltage sensing region S4 is denoted with +++

Figure 7

Figure 7. Modification of thiol groups on the human long NT isoform of $Ca_v1.2$ alters function. (A) Representative single-channel currents recorded at -100 mV in the absence and presence of $200 \mu\text{M}$ DTNB followed by 1 mM DTT and then $2 \mu\text{M}$ nisoldipine, including the P_o for each treatment as shown. (B) Means $\pm$ SE of channel open probability for all patches exposed to DTNB, DTT, and nisoldipine (Nisol) as indicated and I-V relationship for currents from experiment (A). * $P < 0.05$. Reproduced with permission from Ref. 82.

Figure 8

Figure 8. Adult guinea pig cardiac myocytes express functional NAD(P)H oxidase but cannot increase superoxide in response to stimulation by angiotensin II. (A) NADPH consumption in the presence of 100 μ M DPI and 500 μ M apocynin (Apo) assessed by spectrophotometry from 5 hearts. (B). Hypoxia is associated with a decrease in cellular superoxide. Dihydroethidium (DHE) fluorescence recorded from a cell exposed to normoxia only and in another cell exposed to normoxia followed by hypoxia (Normoxia/hypoxia); a.u.=arbitrary units. The superoxide scavenger N-tert-butyl-alpha-phenyl-nitrone (PBN) caused 90 $\pm$ 3% decrease in the rate of DHE signal (inset right). (C) Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by hypoxia

(Normoxia/hypoxia). (D) DHE fluorescence recorded from a cell exposed to hypoxia only and in another cell exposed to hypoxia followed by 5 μ M angiotensin II (Ang II) (Hypoxia/Ang II). Ratio of fluorescence for cells exposed to hypoxia only and for cells exposed to hypoxia followed by Ang II (Hypoxia/Ang II) is shown inset right. (E). Ang II does not alter superoxide during exposure to room oxygen in cardiac myocytes. DHE fluorescence recorded from a cell exposed to normoxia only and in another cell exposed to normoxia followed by addition of 5 μ M Ang II at 30 min (Normoxia/Ang II). Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by Ang II (Normoxia/Ang II) is shown inset right. (F) Ang II increases cellular superoxide in embryonic cortical neurons. DHE fluorescence recorded from a neuron exposed to normoxia only and in another neuron exposed to normoxia followed by 5 μ M Ang II (Normoxia/Ang II). Ratio of fluorescence for cells exposed to normoxia only and for cells exposed to normoxia followed by Ang II (Normoxia/Ang II) is shown inset at right. Reproduced with permission from Ref. 106.

Figure 9

Figure 9. Representative experimental traces demonstrating the effects of hypoxia and β -adrenergic receptor stimulation on action potentials (AP) paced at 1000-ms cycle length in a guinea pig ventricular myocyte.

(A) Effect of reducing pO_2 from 150 mm Hg (normoxia) to 17 mm Hg (hypoxia) on the AP. (B) Effect of hypoxia + 3 nM isoproterenol (β -adrenergic receptor agonist) on the AP. (C) Progression to early afterdepolarization formation in the same cell. (D) Progression to sustained activity in the same cell. In D, the myocyte was not stimulated and APs were spontaneous. See text for further detail. Reproduced with permission from Ref.129.