Eur Biophys J (2005) 34: 369–376
DOI 10.1007/s00249-005-0462-3

ARTICLE

Livia C. Hool

Acute hypoxia differentially regulates K^+ channels. Implications with respect to cardiac arrhythmia

Received: 10 November 2004 / Revised: 3 January 2005 / Accepted: 14 January 2005 / Published online: 22 February 2005

Abstract The first ion channels demonstrated to be sensitive to changes in oxygen tension were K^+ channels in glomus cells of the carotid body. Since then a number of hypoxia-sensitive ion channels have been identified. However, not all K^+ channels respond to hypoxia alike. This has raised some debate about how cells detect changes in oxygen tension. Because ion channels respond rapidly to hypoxia it has been proposed that the channel is itself an oxygen sensor. However, channel function can also be modified by thiol reducing and oxidizing agents, implicating reactive oxygen species as signals in hypoxic events. Cardiac ion channels can also be modified by hypoxia and redox agents. The rapid and slow components of the delayed rectifier K^+ channel are differentially regulated by hypoxia and β-adrenergic receptor stimulation. Mutations in the genes that encode the subunits for the channel are associated with Long QT syndrome and sudden cardiac death. The implications with respect to effects of hypoxia on the channel and triggering of cardiac arrhythmia will be discussed.

Keywords K^+ channels · Hypoxia · β-adrenergic regulation · Long QT syndrome · Oxygen sensing

Introduction

All cells have the necessary mechanisms to adapt and respond to a hypoxic environment to prevent the deleterious effects of prolonged oxygen deprivation. It has been recognized for some years that the carotid bodies act as sensors to detect changes in arterial oxygen tension, pH and CO_2. They respond by increasing discharge levels of afferent chemosensory fibres in the carotid sinus nerve that alert the cardiorespiratory centre in the brain, resulting in reflex changes in cardiovascular and respiratory function (Lopez-Lopez et al. 1989). Central to this adaptive response are K^+ channels. The activation of transmitter release from chemoreceptor cells occurs as a result of inhibition of delayed rectifier K^+ channels by hypoxia (Lopez-Barneo et al. 1988). This was the first indication that ion channels are responsive to changes in oxygen tension. Although the molecular and pharmacological identity of the K^+ channel varies, several studies have supported the existence of hypoxia-sensitive K^+ channels in chemoreceptor cells of the carotid body (Delpiano and Hescheler 1989, Lopez-Lopez et al. 1989, Peers 1990, Stea and Nurse 1991, Buckler 1997) aorta (Ito et al. 1999), lung (Youngson et al. 1993) and adrenal medulla (Thompson and coworkers 1997, 2002, Zhu et al. 1996, Mochizuki-Oda et al. 1997).

In addition to studies in chemoreceptor cells, it is now recognized that a number of cell types express K^+ channels that are responsive to hypoxia. Hypoxic pulmonary vasoconstriction is a mechanism that prevents ventilation/perfusion mismatch by diverting blood away from poorly ventilated regions of the lung. Resistance pulmonary arteries serving the hypoxic lobes constrict, while the larger pulmonary arteries are unresponsive or dilate (Weir and Archer 1995). One view of this process is that hypoxia induces vasoconstriction by regulating cyclic adenosine diphosphate–ribose production that mobilizes calcium from ryanodine-sensitive sarcoplasmic reticulum stores. Pulmonary vasoconstriction then progresses as a result of release of vasoconstrictors from the endothelium (Evans and Dipp 2002). Another view is that resistance pulmonary artery smooth muscle cells preferentially express 4-aminopyridine and correolide-sensitive K^+ channels (in particular Kv1.5 protein) that are also hypoxia-responsive (Archer et al. 2004). In these cells hypoxia causes inhibition of K^+ channels, leading to membrane depolarization, influx of Ca^{2+} through voltage-dependent channels and myocyte contraction.

L. C. Hool
Physiology M311, School of Biomedical and Chemical Sciences, Australia and The Western Australian Institute of Medical Research, The University of Western Australia,
35 Stirling Highway, Crawley, WA, 6009, Australia
E-mail: lhool@cyllene.uwa.edu.au
Tel.: +61-86-4883307
Fax: +61-86-4881025

However, not all K^+ channels are inhibited during hypoxia. In fact the mechanism by which the ductus arteriosus contracts at the moment the newborn breathes the first breath is proposed to involve oxygen-sensitive K^+ channels that are inhibited by normoxia (Michelakis et al. 2002b, Thebaud et al. 2002). In systemic arteries and cerebral vessels, hypoxia increases K^+ currents that cause vasodilation in an attempt to increase blood supply to poorly perfused tissues. Similarly, in renal arteries an increase in outward K^+ channel current is associated with dilation of the arteries during hypoxia (Michelakis et al. 2002a) and where hypoxia is severe enough to compromise production of ATP, opening of K_{ATP} channels contributes to smooth muscle relaxation (Mellemkjaer and Nielsen-Kudsk 1994, Gasser et al. 1993).

Although the responses may vary, it is clear that a number of K^+ channels can respond to alteration in oxygen tension. The physiological consequence of altered channel function reflects the cell or tissue's attempt to restore homeostasis. In some cell types, however, adaptive responses are not adequate to prevent cell damage even during transient episodes of hypoxia. This is particularly apparent in the cardiovascular system, where a lack of oxygen contributes to myocardial infarction and cardiac arrhythmia (Keating and Sanguinetti 2001, Sanguinetti 2002). The role of oxygen in myocardial function has been studied for many years; however, only recently a greater understanding is emerging about the effects of changes in oxygen tension on cardiac ion channels. This article will present what is currently known about the effect of hypoxia on cardiac K^+ channels and the role of ion channels in cellular oxygen sensing. Because acute (minutes) and chronic (hours–days) hypoxia result in different responses, this article will discuss the role of K^+ channels during acute hypoxic events only. Some evidence will be presented to show that hypoxia and modification of the cell's redox state can alter the function of the slow component of the cardiac delayed rectifier K^+ channel.

The K^+ channel as an oxygen sensor

A physiological response to hypoxia requires an oxygen sensor and a signal that links the sensor with the functional response. Although the effects of hypoxia on cellular function have been studied for many years, there is little consensus as to the identity of the oxygen sensor. The channel has been considered an oxygen sensor because alterations in channel activity occur very rapidly in excised patches where cytosolic variables such as second messengers, ATP and Ca^{2+} are absent (Ganfornina and Lopez-Barneo 1991, Lopez-Barneo et al. 1988). However the same ion channel type may be inhibited or activated by hypoxia when expressed in different tissue (Peers 1990, Gebremedhin et al. 1994). This may provide some versatility during variations in oxygen but proves difficult to assign a universal oxygen-sensing component to the channel. Although it has not been directly tested, there is no experimental or molecular evidence to suggest that the channel protein is capable of directly sensing changes in oxygen tension. In addition, it is not uncommon for a proportion of hypoxia-sensitive ion channels to be unresponsive to hypoxia (Lopez-Barneo et al. 1997, Riesco-Fagundo et al. 2001). This led to the suggestion that the ion channel may not itself be the oxygen sensor but positioned with or near the oxygen sensor in the plasma membrane. Unresponsive channels may exist because the sensor has been disrupted during cell dissociation or excision of the patch. Since carbon monoxide attenuates hypoxic inhibition of Ca^{2+}-dependent K^+ channels in excised patches, a heme oxygenase has been suggested as a possible sensor (Prabhakar and Overholt 2000, Riesco-Fagundo et al. 2001, Williams et al. 2004). What is likely is that ion channels are effectors responding as a consequence of the signal from the putative sensor. Since reactive oxygen species (ROS) are capable of acting as signalling molecules, possible candidates for oxygen sensing must also include those that are capable of generating ROS. Both NAD(P)H oxidase and the mitochondria are capable of reducing oxygen to produce superoxide. In vascular smooth muscle NAD(P)H oxidase is the primary source of superoxide production (Hanna et al. 2002) but in other cell types it has been argued that the mitochondria are the primary source of generation of ROS (Archer and Michelakis 2002, Michelakis et al. 2002a, Waypa et al. 2001). If ROS are the signal linking the ion channel and the oxygen sensor then ion channel function should be able to be modified by thiol reducing or oxidizing compounds.

Redox agents and K^+ channel function

ROS are important regulators of cellular responses via redox modification of proteins and transcription factors (Gabbita et al. 2000, Wolin 2000). 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 oxygen to superoxide. Superoxide is not a good signalling molecule as it is highly solvent in water and does not cross lipid membranes (Khan and Wilson 1995). However, superoxide is dismutated to H_2O_2, which is 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.

There is good evidence that ROS act as signals linking the oxygen sensor with the channel during hypoxia. Pharmacological agents that alter the cell's redox environment influence ion channel function. Thiol reducing agents such as dithiothreitol mimic the effects of hypoxia on K^+ channels (Weir and Archer 1995, Weir et al. 2002, Park and coworkers 1995a,

1995b, Hool 2004). Although controversial, hypoxia is associated with a decrease in cellular superoxide and H_2O_2 (Weir and Archer 1995, Weir et al. 2002, Kroll and Czyzyk-Krzeska 1998, Hanson and Leibold 1998, Michelakis et al. 2002b, Hool and Arthur 2002). This is controversial because it has also been suggested that cellular ROS increase during hypoxia (Chandel and Schumacker 2000, Duranteau et al. 1998, Waypa et al. 2001). Under hypoxic conditions, it has been proposed that mitochondrial electron transfer by cytochrome oxidase becomes inhibited and an increase in the generation of superoxide occurs owing to electron transfer to oxygen by ubisemiquinone. This model has been used to explain O_2-sensing in contracting embryonic chick cardiomyocytes. An important consideration here, however, is that the shift in V_{max} for cytochrome oxidase is dependent upon the available oxygen, the pool of ubisemiquinone, the reduced state of the electron transfer proteins, the mitochondrial membrane potential and limitations regarding the measurement of superoxide production (Korshunov et al. 1997, Swift and Sarvazyan 2000, Turrens and Boveris 1980). Increases in ROS production were not evident until 1–2 h after induction of hypoxia in the chick cardiomyocytes, confirming that the duration of hypoxia is important in determining the mechanisms of ROS production (Becker et al. 1999, vanden Hoek et al. 1998). Acute exposure to hypoxia (less than 1 h) 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 versus 2–3 mmHg) (Casey et al. 2002). Consistent therefore with a decrease in cellular ROS during acute hypoxia, perfusing myocytes intracellular with catalase (that specifically converts H_2O_2 to H_2O and oxygen) has been shown to mimic the hypoxic response (Hool and Arthur 2002).

However, thiol reducing agents do not always mimic the hypoxic response (Reeve et al. 2001, Roy et al. 2001, Park and coworkers 1995a, 1995b). The response may be the sum effect of nondiscriminatory targeting of thiol groups by the drug. It may also be consistent with the same type of ion channel responding differentially to hypoxia. For example, a delayed rectifier K^+ channel is inhibited by hypoxia in pulmonary artery smooth muscle cells but is activated by hypoxia in renal and cerebral arteries (see "Introduction"). The reasons for differential responses are unclear. At least for pulmonary and renal arteries it has been postulated that the differential response may be due to diversity in mitochondrial function and production of ROS (Michelakis et al. 2002a). Nevertheless these studies confirm that K^+ channel function can be altered by changes in the redox state of the cell and there is an association between altered cellular production of ROS during hypoxia and channel responses.

Role of K^+ channels in cardiac excitability

Repolarization of the cardiac action potential is dependent upon the activity of a number of K^+ channels (Fig. 1). The slow and fast transient outward K^+ currents are responsible for the early phase (phase 1) of repolarization, while delayed outwardly rectifying K^+ channels are responsible for late (phase 3) repolarization. These include the rapid (I_{Kr}) and slow components (I_{Ks}) of the delayed rectifier K^+ channel and the inwardly rectifying K^+ channel (I_{K1}) that determines the cell's resting membrane potential. These channels, with the exception of the I_{K1} channel are members of the Kv family of ion channels and like most channels are heteromeric proteins comprising four pore-forming (α) subunits and an accessory β subunit. Each Kv α subunit encodes a protein with six transmembrane domains, a voltage-sensing (S4) domain and a P loop that comprises the K^+ selective pore.

Functional cardiac I_{Kr} channels require the expression of a minK (KCNE1 β subunit) or a minK-related peptide (MiRP1) and the human ether-a-go-go-related gene (HERG) complex (Abbott et al. 1999). I_{Kr} is characterized by its sensitivity to be blocked by lanthanum and benzenesulfonamide antiarrhythmics. In addition, it activates very rapidly and typically exhibits prominent rectification (Sanguinetti and Jurkiewicz 1990). On the other hand, I_{Ks} is characterized by a delayed onset of activation that can be blocked by chromanol and is encoded by the genes, KvLQT1 (KCNQ1) and KCNE1 (McDonald et al. 1997, Sanguinetti et al. 1996, Barhanin et al. 1996). Mutations in KvLQT1, HERG and KCNE1 have been linked to familial Long QT (LQT) syndromes, whereby a lengthening of the QT interval leads to increased risk of *torsades de pointes* and sudden cardiac death (Keating and

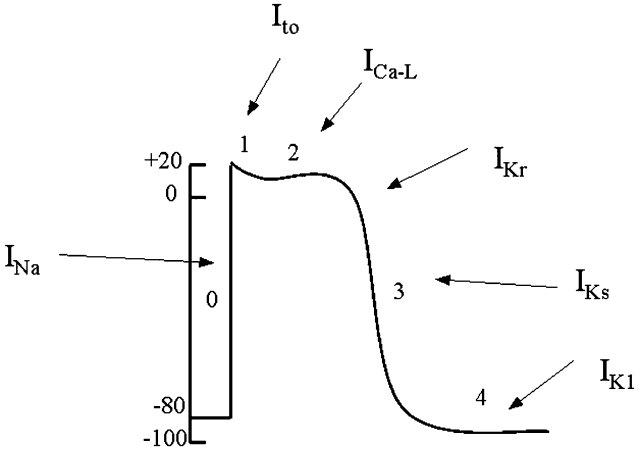

Fig. 1 Schematic showing the activation phase (*0*), the plateau phase (*2*) and the repolarization phases (*1*, *3*, *4*) of the cardiac action potential. The points at which the fast inward Na^+ current (I_{Na}), the L-type Ca^{2+} current ($I_{Ca\text{-}L}$), the transient outward K^+ current (I_{to}) and the rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier K^+ current are active are indicated (see text for detail)

Sanguinetti 2001, Splawski et al. 1997). Patients with mutations in KvLQT1 typically develop arrhythmias under emotional stress when the adrenergic system is activated (Schwartz et al. 2001).

A number of studies have been undertaken characterizing electrophysiological properties of HERG coexpressed with or without minK and/or MiRP1 (Tseng 2001) in heterologous systems. However, a true characterization of the channel can only be found when it is studied in its native state assembled with α and auxiliary subunits. Despite considerable characterization of the effects of hypoxia on K^+ channels in noncardiac tissue, until recently no studies had been performed that examined the effects of acute hypoxia on the cardiac delayed rectifier K^+ channel. Interestingly, the slow and rapid components of the channel are differentially regulated by hypoxia and redox state.

Hypoxia and K^+ channels in the heart

The first K^+ channel that was found to be modulated by hypoxia in the heart was the ATP-sensitive K^+ channel. It was first described as an outward current that activated upon exposure to cyanide and decreased when ATP was applied (Noma 1983). Hypoxia (presumably at a level that caused metabolic inhibition) activated the channels. Since then a number of hypoxia-sensitive channels have been identified in cardiac myocytes (Fearon and coworkers 1997, 1999, 2000, Ju et al. 1994, 1996, Hool 2000, 2001, Hool and Arthur 2002). The effect of hypoxia on cardiac HERG or the native I_{Ks} or I_{Kr} has not been previously studied. We decided to examine the effect of acute hypoxia on both the slow and the rapid components of the delayed rectifier K^+ channel in the absence and presence of β-adrenergic receptor stimulation in isolated guinea pig ventricular myocytes (Hool 2004). Understanding the effects of adrenergic stimulation on channel function during hypoxia is important because of the association between emotional stress and the triggering of arrhythmias in patients with mutations in KvLQT1. Hypoxia inhibited basal I_{Ks} by way of redox modification of the channel protein because the thiol reducing agent dithiothreitol mimicked the effect of hypoxia and oxidizing agents such as 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) attenuated the effect of hypoxia. In addition, hypoxia increased the sensitivity of I_{Ks} to β-adrenergic receptor stimulation (Fig. 2). This effect could also be mimicked by the thiol reducing agent dithiothreitol. However, the site of thiol group modification probably differs from that involved in basal inhibition of the current by hypoxia alone because the membrane-impermeant oxidizing agent DTNB did not alter the sensitivity of the channel to isoproterenol during hypoxia.

In contrast, hypoxia was without an effect on I_{Kr} (Fig. 3). It is likely that the absence of an effect of hypoxia on I_{Kr} is because channel function cannot be modulated by redox modification of thiol groups. Consistent with this we could not record any effect of dithiothreitol or DTNB on I_{Kr}. In addition, dithiothreitol, hydrogen peroxide, [2-(trimethylammonium)ethyl] methanethiosulfonate (MTSET) and (2-sulfonatoethyl)methanethiosulfonate (MTSES) are without effect on HERG channels expressed in xenopus oocytes (Fan et al. 1999, Dun et al. 1999). We also found that I_{Kr} could not be modulated by β-adrenergic receptor stimulation (Fig. 3). The HERG channel contains a segment homologous to a cyclic nucleotide binding domain (Warmke and Ganetzky 1994) and at least four putative phosphorylation sites for protein kinase A (Cui et al. 2000). However, consistent with our results, agonists that increase protein kinase A or cAMP activity have been reported to have no effect (Sanguinetti and coworkers 1991, 1995) or an inhibition of native I_{Kr} at nonphysiological concentrations of isoproterenol (1–10 μM) (Karle et al. 2002).

The availability of accessory subunits and sulfhydryl groups may be necessary for a response. It is known that minK forms I_{Ks} channels in xenopus oocytes with an endogenous xenopus subunit KvLQT1 (Sanguinetti et al. 1996). In addition, modification of minK activity by the thiol oxidizing agent thiomerosal requires an additional sulfhydryl group on an auxiliary subunit (Yao et al. 1997). MinK also appears to regulate the response of HERG to cAMP and protein kinase A. When HERG is expressed alone, cAMP and protein kinase A act in parallel to inhibit HERG current. However, this effect is negated when HERG is complexed with minK and a hyperpolarizing shift in voltage-dependent activation of the current becomes predominant (Cui et al. 2000).

Unanswered questions and future directions

It is clear that not all ion channels respond to hypoxia alike. This highlights the importance of careful characterization of the effect of hypoxia on cardiac ion channels but with the caveat that the level of oxygen tension and the duration of hypoxia will be important in defining responses. Even acute anoxia associated with cellular metabolic inhibition is likely to result in differential responses compared with acute hypoxia at a level of oxygen tension that does not depress metabolic function and ATP production. In addition, longer-term hypoxia will alter ion channel expression and cellular excitability as a result of the induction of hypoxia-responsive genes (Peers 2002). Therefore it is important that the level and duration of hypoxia is clearly defined if mechanistic insight into effects of acute hypoxia that mimic the hypoxic event in vivo is to be gained.

Probably the most curious observation is that I_{Kr} is unresponsive to hypoxia even though HERG contains a PAS (acronym for the gene products of Per, Arnt and Sim) domain that would be expected to be responsive to hypoxia or redox agents (Gu et al. 2000). It is not known whether recombinant cardiac HERG alone can respond to hypoxia. It may be necessary to establish whether the

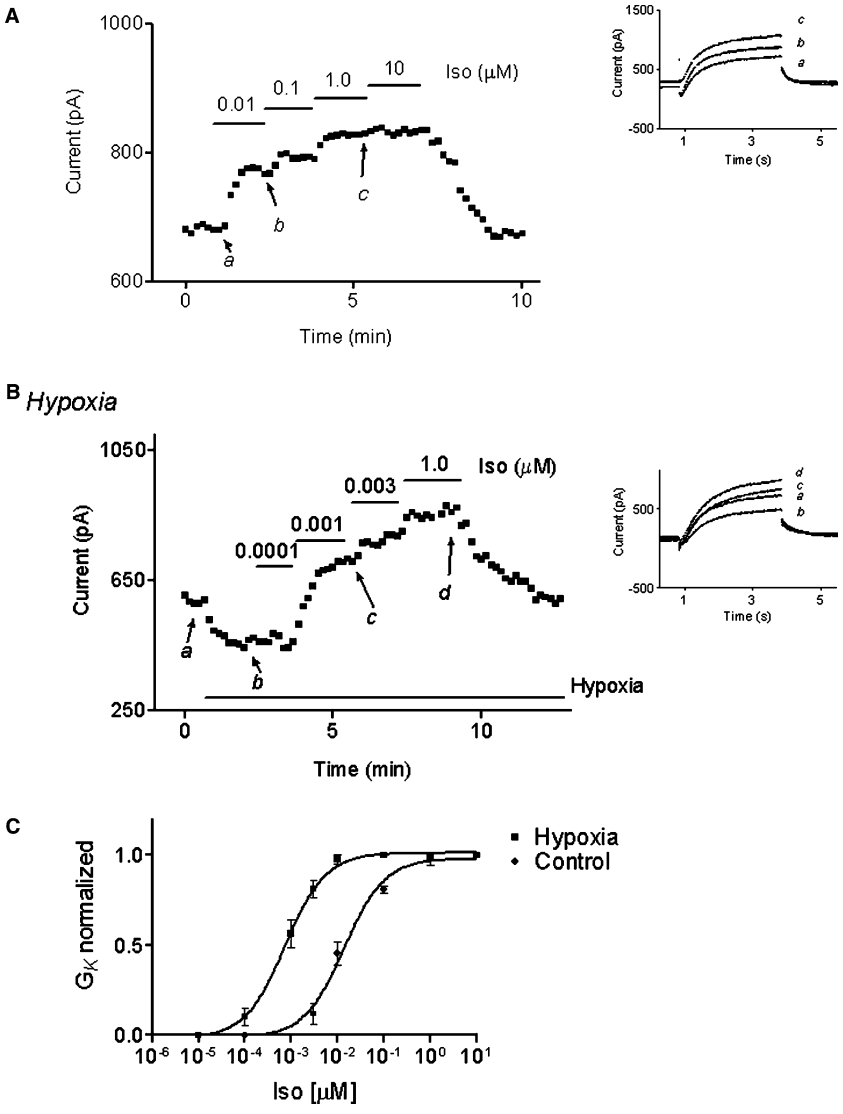

Fig. 2 Hypoxia (*Hyp*) increases the sensitivity of I_{Ks} to β-adrenergic receptor stimulation. Time course of changes in membrane current recorded in a cell during exposure to increasing concentrations of isoproterenol (*Iso*) in the absence (**a**) and presence (**b**) of Hyp, including membrane currents recorded at the time points indicated (*inset*). **c** Concentration-dependence of Iso activation of I_{Ks} in the absence ($n=4$–8 at each data point) and presence ($n=4$–9 at each data point) of Hyp. Exposure to 1 μM Iso represented a maximally stimulating concentration of the agonist. The K^+ conductance (G_K) measured at each concentration of Iso was normalized to G_K measured in the presence of 1 μM Iso in the same cell. Data were fit to a logistic equation using a nonlinear least-squares curve-fitting routine (GraphPad Prism). Modified from Hool (2004)

subunits alone are responsive to hypoxia and whether an accessory subunit or an intermediate protein modulates a response. A hypoxia-sensitive HERG-like channel has been reported in the carotid body (Overholt et al. 2000) and in neuroblastoma cells exposed to severe hypoxia long-term (4–7 days) (Fontana et al. 2001). If cardiac HERG alone is responsive to hypoxia and minK or MiRP modifies the response by HERG to hypoxia, this may provide important insight into the regulation of the pore-forming ion channel subunit by accessory proteins. In addition, further understanding the mechanisms of oxygen sensing and signalling during hypoxia in cardiac myocytes may explain why some channels are unresponsive to hypoxia.

Clinical relevance of β-adrenergic regulation of cardiac K^+ channels during hypoxia

β-adrenergic receptor blocking drugs have been used routinely as treatment for patients with LQT syndrome with variable success (Moss et al. 2000). Understanding the net effect of hypoxia on cardiac ion channels is necessary when considering treatment options. Both components of I_K play a significant role in cardiac repolarization. It has been proposed that an important function of I_{Ks} is to counteract the excessive prolongation of the action potential duration induced by β-adrenergic enhancement of the L-type Ca^{2+} channel (Han et al. 2001). Enhancement of the L-type Ca^{2+} channel promotes arrhythmogenic after depolarizations (Marban et al. 1986, January and Riddle 1989). Hypoxia also increases the sensitivity of the L-type Ca^{2+} channel to β-adrenergic receptor stimulation (Hool 2000, 2001, Hool and Arthur 2002) and increases a persistently inactivating Na^+ current in the heart (Ju et al. 1994, 1996). Since I_{Ks} could be modified by hypoxia and β-adrenergic receptor activation, these data provide some insight into the relative risk of arrhythmia in patients with LQT syndrome. A patient with a mutation in KvLQT1 or KCNE1 may be at greater risk of arrhythmia during a hypoxic event and emotional stress

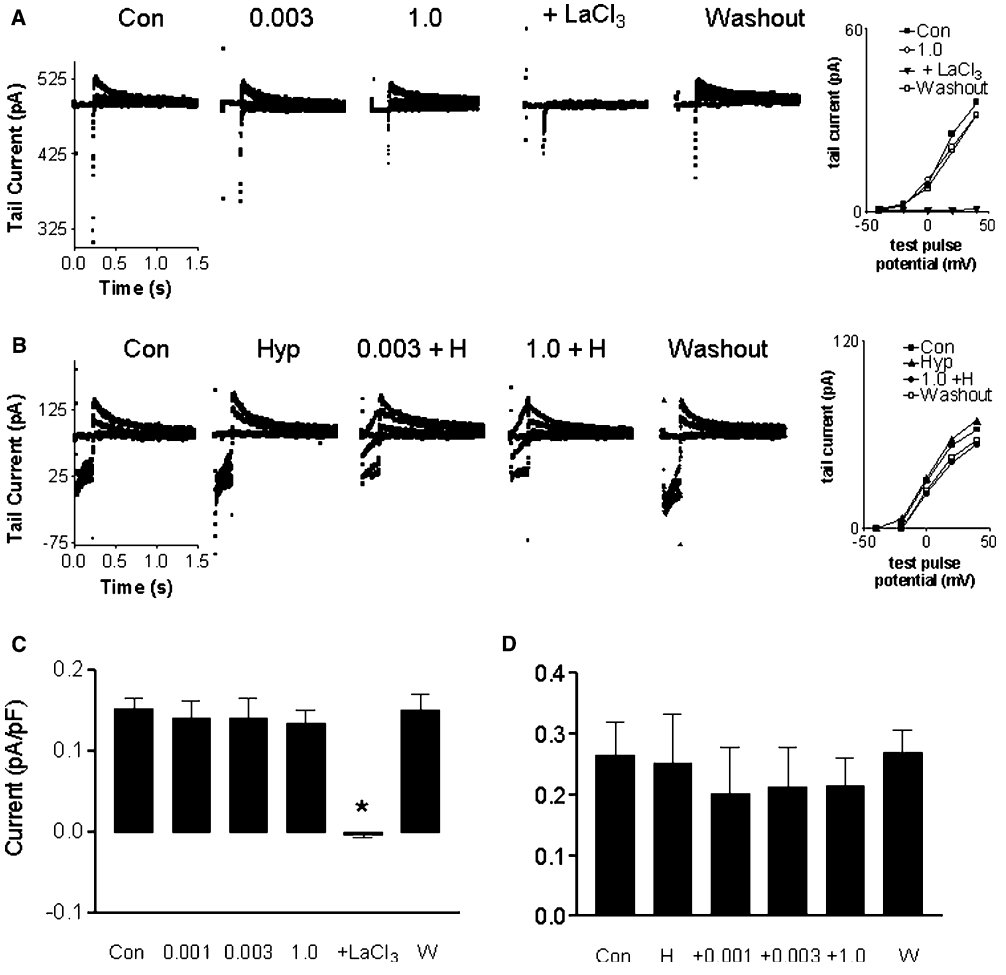

Fig. 3 Hyp and Iso are without effect on I_{Kr}. **a** I_{Kr} tail currents in the presence of 0.003 μM Iso (*0.003*), 1.0 μM Iso (*1.0*) and 1 μM Iso + 10 μM $LaCl_3$ (*+LaCl₃*). Measurements were taken every 2 min. **b** I_{Kr} tail currents elicited in the presence of Hyp and 0.003 μM Iso or 1.0 μM Iso + Hyp (*+H*). **c** Mean ± standard error (*SE*) of I_{Kr} tail currents in nine cells measured at +40 mV in the control and during exposure to increasing concentrations of Iso as indicated in micromoles per litre, and 1.0 μM Iso + $LaCl_3$ (*+LaCl₃*). **d** Mean ± SE of I_{Kr} tail currents in seven cells measured at +40 mV in the control and during exposure to Hyp alone (*H*) and Hyp plus increasing concentrations of Iso (in micromoles per litre) as indicated. *W* washout

than a patient with a mutation in HERG alone. Characterizing and understanding these responses may further help clinicians with patient selection for β-blockade treatment.

Acknowledgements This study was supported by the National Health and Medical Research Council of Australia. L.C.H. is a National Health and Medical Research Career Development Award Fellow.

References

Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97:175–187

Archer S, Michelakis E (2002) The mechanism(s) of hypoxic pulmonary vasoconstriction: potassium channels, redox O(2) sensors, and controversies. News Physiol Sci 17:131–137

Archer SL, Wu XC, Thebaud B, Nsair A, Bonnet S, Tyrrell B, McMurtry MS, Hashimoto K, Harry G, Michelakis ED (2004) Preferential expression and function of voltage-gated, O_2-sensitive K+ channels in resistance pulmonary arteries explains regional heterogeneity in hypoxic pulmonary vasoconstriction: ionic diversity in smooth muscle cells. Circ Res 95:308–318

Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G (1996) K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. Nature 384:78–80

Becker LB, vanden Hoek TL, Shao ZH, Li CQ, Schumacker PT (1999) Generation of superoxide in cardiomyocytes during ischemia before reperfusion. Am J Physiol 277:H2240–H2246

Buckler KJ (1997) A novel oxygen-sensitive potassium current in rat carotid body type I cells. J Physiol 498:649–662

Casey TM, Pakay JL, Guppy M, Arthur PG (2002) Hypoxia causes downregulation of protein and RNA synthesis in noncontracting Mammalian cardiomyocytes. Circ Res 90:777–783

Chandel NS, Schumacker PT (2000) Cellular oxygen sensing by mitochondria: old questions, new insight. J Appl Physiol 88:1880–1889

Cui J, Melman Y, Palma E, Fishman GI, McDonald TV (2000) Cyclic AMP regulates the HERG K(+) channel by dual pathways. Curr Biol 10:671–674

Delpiano MA, Hescheler J (1989) Evidence for a PO2-sensitive K+ channel in the type-I cell of the rabbit carotid body. FEBS Lett 249:195–198

Dun W, Jiang M, Tseng GN (1999) Allosteric effects of mutations in the extracellular S5-P loop on the gating and ion permeation properties of the hERG potassium channel. Pfluggers Arch:Eur J Physiol 439:141–149

Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT (1998) Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J Biol Chem 273:11619–11624

Evans AM, Dipp M (2002) Hypoxic pulmonary vasoconstriction: cyclic adenosine diphosphate-ribose, smooth muscle Ca(2+) stores and the endothelium. Respir Physiol Neurobiol 132:3–15

Fan JS, Jiang M, Dun W, McDonald TV, Tseng GN (1999) Effects of outer mouth mutations on hERG channel function: a comparison with similar mutations in the Shaker channel. Biophys J 76:3128–3140

Fearon IM, Palmer AC, Balmforth AJ, Ball SG, Mikala G, Schwartz A, Peers C (1997) Hypoxia inhibits the recombinant alpha 1C subunit of the human cardiac L-type Ca2+ channel. J Physiol 500:551–556

Fearon IM, Palmer AC, Balmforth AJ, Ball SG, Varadi G, Peers C (1999) Modulation of recombinant human cardiac L-type Ca2+ channel alpha1C subunits by redox agents and hypoxia. J Physiol 514:629–637

Fearon IM, Varadi G, Koch S, Isaacsohn I, Ball SG, Peers C (2000) Splice variants reveal the region involved in oxygen sensing by recombinant human L-type Ca^{2+} channels. Circ Res 87:537–539

Fontana L, D'Amico M, Crociani O, Biagiotti T, Solazzo M, Rosati B, Arcangeli A, Wanke E, Olivotto M (2001) Long-term modulation of HERG channel gating in hypoxia. Biochem Biophys Res Co 286:857–862

Gabbita SP, Robinson KA, Stewart CA, Floyd RA, Hensley K (2000) Redox regulatory mechanisms of cellular signal transduction. Arch Biochem Biophys 376:1–13

Ganfornina MD, Lopez-Barneo J (1991) Single K+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension. Proc Natl Acad Sci USA 88:2927–2930

Gasser R, Klein W, Kickenweiz E (1993) Vasodilative response to hypoxia and simulated ischemia is mediated by ATP-sensitive K+ channels in guinea pig thoracic aorta. Angiology 44:228–243

Gebremedhin D, Bonnet P, Greene AS, England SK, Rusch NJ, Lombard JH, Harder DR (1994) Hypoxia increases the activity of Ca(2+)-sensitive K+ channels in cat cerebral arterial muscle cell membranes. Pfluggers Arch: Eur J Physiol 428:621–630

Gu YZ, Hogenesch JB, Bradfield CA (2000) The PAS superfamily: sensors of environmental and developmental signals. Annu Rev Pharmacol 40:519–561

Han W, Wang Z, Nattel S (2001) Slow delayed rectifier current and repolarization in canine cardiac Purkinje cells. Am J Physiol 280:H1075–H1080

Hanna IR, Taniyama Y, Szocs K, Rocic P, Griendling KK (2002) NAD(P)H oxidase-derived reactive oxygen species as mediators of angiotensin II signaling. Antioxid Redox Signal 4:899–914

Hanson ES, Leibold EA (1998) Regulation of iron regulatory protein 1 during hypoxia and hypoxia/reoxygenation. J Biol Chem 273:7588–7593

Hool LC (2000) 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 87:1164–1171

Hool LC (2001) 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 88:1036–1043

Hool LC (2004) Differential regulation of the slow and rapid components of guinea-pig cardiac delayed rectifier K^+ channels by hypoxia. J Physiol 554:743–754

Hool LC, Arthur PG (2002) Decreasing cellular hydrogen peroxide with catalase mimics the effects of hypoxia on the sensitivity of the L-type Ca2+ channel to beta-adrenergic receptor stimulation in cardiac myocytes. Circ Res 91:601–609

Ito S, Ohta T, Nakazato Y (1999) Characteristics of 5-HT-containing chemoreceptor cells of the chicken aortic body. J Physiol 515:49–59

January CT, Riddle JM (1989) Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca2+ current. Circ Res 64:977–990

Ju YK, Saint DA, Gage PW (1994) Inactivation-resistant channels underlying the persistent sodium current in rat ventricular myocytes. Proc R Soc Lond 256:163–168

Ju YK, Saint DA, Gage PW (1996) Hypoxia increases persistent sodium current in rat ventricular myocytes. J Physiol 497:337–347

Karle CA, Zitron E, Zhang W, Kathofer S, Schoels W, Kiehn J (2002) Rapid component I(Kr) of the guinea-pig cardiac delayed rectifier K(+) current is inhibited by beta(1)-adrenoreceptor activation, via cAMP/protein kinase A-dependent pathways. Cardiovasc Res 53:355–362

Keating MT, Sanguinetti MC (2001) Molecular and cellular mechanisms of cardiac arrhythmias. Cell 104:569–580

Khan AU, Wilson T (1995) Reactive oxygen species as cellular messengers. Chem Biol 2:437–445

Korshunov SS, Skulachev VP, Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416:15–18

Kroll SL, Czyzyk-Krzeska MF (1998) Role of H2O2 and heme-containing O_2 sensors in hypoxic regulation of tyrosine hydroxylase gene expression. Am J Physiol 274:C167–C174

Lopez-Barneo J, Lopez-Lopez JR, Urena J, Gonzalez C (1988) Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. Science 241:580–582

Lopez-Barneo J, Ortega-Saenz P, Molina A, Franco-Obregon A, Urena J, Castellano A (1997) Oxygen sensing by ion channels. Kidney Int 51:454–461

Lopez-Lopez J, Gonzalez C, Urena J, Lopez-Barneo J (1989) Low pO2 selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. J Gen Physiol 93:1001–1015

Marban E, Robinson SW, Wier WG (1986) Mechanisms of arrhythmogenic delayed and early afterdepolarizations in ferret ventricular muscle. J Clin Invest 78:1185–1192

McDonald TV, Yu Z, Ming Z, Palma E, Meyers MB, Wang KW, Goldstein SA, Fishman GI (1997) A minK-HERG complex regulates the cardiac potassium current I(Kr). Nature 388:289–292

Mellemkjaer S, Nielsen-Kudsk JE (1994) Glibenclamide inhibits hypoxic relaxation of isolated porcine coronary arteries under conditions of impaired glycolysis. Eur J Pharmacol 270:307–312

Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, Gurtu R, Archer SL (2002a) Diversity in mitochondrial function explains differences in vascular oxygen sensing. Circ Res 90:1307–1315

Michelakis ED, Rebeyka I, Wu X, Nsair A, Thebaud B, Hashimoto K, Dyck JR, Haromy A, Harry G, Barr A, Archer SL (2002b) O_2 sensing in the human ductus arteriosus: regulation of voltage-gated K+ channels in smooth muscle cells by a mitochondrial redox sensor. Circ Res 91:478–486

Mochizuki-Oda N, Takeuchi Y, Matsumura K, Oosawa Y, Watanabe Y (1997) Hypoxia-induced catecholamine release and intracellular Ca^{2+} increase via suppression of K+ channels in cultured rat adrenal chromaffin cells. J Neurochem 69:377–387

Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, Medina A, Zhang L, Robinson JL, Timothy K, Towbin JA, Andrews ML (2000) Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. Circulation 101:616–623

Noma A (1983) ATP-regulated K+ channels in cardiac muscle. Nature 305:147–148

Overholt JL, Ficker E, Yang T, Shams H, Bright GR, Prabhakar NR (2000) Chemosensing at the carotid body. Involvement of a HERG-like potassium current in glomus cells. Adv Exp Med Biol 475:241–248

Park MK, Lee SH, Ho WK, Earm YE (1995a) Redox agents as a link between hypoxia and the responses of ionic channels in rabbit pulmonary vascular smooth muscle. Exp Physiol 80:835–842

Park MK, Lee SH, Lee SJ, Ho WK, Earm YE (1995b) Different modulation of Ca-activated K channels by the intracellular redox potential in pulmonary and ear arterial smooth muscle cells of the rabbit. Pfluggers Arch: Eur J Physiol 430:308–314

Peers C (1990) Hypoxic suppression of K+ currents in type I carotid body cells: selective effect on the Ca2(+)-activated K+ current. Neurosci Lett 119:253–256

Peers C (2002) The G. L. Brown Prize lecture. Hypoxic regulation of ion channel function and expression. Exp Physiol 87:413–422

Prabhakar NR, Overholt JL (2000) Cellular mechanisms of oxygen sensing at the carotid body: heme proteins and ion channels. Respir Physiol 122:209–221

Reeve HL, Tolarova S, Nelson DP, Archer S, Weir EK (2001) Redox control of oxygen sensing in the rabbit ductus arteriosus. J Physiol 533:253–261

Riesco-Fagundo A, Perez-Garcia M, Gonzalez C, Lopez-Lopez JR (2001) O_2 modulates maxiK channels of rat chemoreceptor cells by a membrane restricted and CO sensitive mechanism. Circ Res 89:430–436

Roy A, Mokashi A, Rozanov C, Daudu PA, Lahiri S (2001) Reduced glutathione, dithiothreitol and cytochrome P-450 inhibitors do not influence hypoxic chemosensory responses in the rat carotid body. Brain Res 889:131–137

Sanguinetti MC (2002) When the KChIPs are down. Nat Med 8:18–19

Sanguinetti MC, Jurkiewicz NK (1990) Two components of cardiac delayed rectifier K + current. Differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 96:195–215

Sanguinetti MC, Jurkiewicz NK, Scott A, Siegl PK (1991) Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. Circ Res 68:77–84

Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 81:299–307

Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT (1996) Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature 384:80–83

Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R (2001) Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 103:89–95

Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, eating MT (1997) Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nat Gen 17:338–340

Stea A, Nurse CA (1991) Whole-cell and perforated-patch recordings from O2-sensitive rat carotid body cells grown in short-term and long-term culture. Pfluggers Arch: Eur J Physiol 418:93–101

Swift LM, Sarvazyan N (2000) Localization of dichlorofluorescin in cardiac myocytes: implications for assessment of oxidative stress. Am J Physiol 278:H982–H990

Thebaud B, Michelakis E, Wu XC, Harry G, Hashimoto K, Archer SL (2002) Sildenafil reverses O2 constriction of the rabbit ductus arteriosus by inhibiting type 5 phosphodiesterase and activating BK(Ca) channels. Pediatr Res 52:19–24

Thompson RJ, Jackson A, Nurse CA (1997) Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells. J Physiol 498:503–510

Thompson RJ, Farragher SM, Cutz E, Nurse CA (2002) Developmental regulation of O(2) sensing in neonatal adrenal chromaffin cells from wild-type and NADPH-oxidase-deficient mice. Pfluggers Arch :Eur J Physiol 444:539–548

Tseng GN (2001) I(Kr): the hERG channel. J Mol Cell Cardiol 33:835–849

Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427

vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT (1998) Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem 273:18092–18098

Warmke JW, Ganetzky B (1994) A family of potassium channel genes related to eag in Drosophila and mammals. Proc Natl Acad Sci USA 91:3438–3442

Waypa GB, Chandel NS, Schumacker PT (2001) Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ Res 88:1259–1266

Weir EK, Archer SL (1995) The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. FASEB J 9:183–189

Weir EK, Hong Z, Porter VA, Reeve HL (2002) Redox signaling in oxygen sensing by vessels. Respir Physiol Neurobiol 132:121–130

Williams SEJ, Wootton P, Mason HS, Bould J, Iles DE, Riccardi D, Peers C, Kemp PJ (2004) Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. Science 306:2093–2097

Wolin MS (2000) Interactions of oxidants with vascular signaling systems. Arterioscler Thromob Vasc 20:1430–1442

Yao JA, Jiang M, Tseng GN (1997) Mechanism of enhancement of slow delayed rectifier current by extracellular sulfhydryl modification. Am J Physiol 273:H208–H219

Youngson C, Nurse C, Yeger H, Cutz E (1993) Oxygen sensing in airway chemoreceptors. Nature 365:153–155

Zhu WH, Conforti L, Czyzyk-Krzeska MF, Millhorn DE (1996) Membrane depolarization in PC-12 cells during hypoxia is regulated by an O_2-sensitive K + current. Am J Physiol 271:C658–C665