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Dear Dr Scheid:

We have been very pleased to accept Drs. Kumar and Prabhakar invitation to contribute to a Special Issue on the physiology and pathophysiology of the carotid body. Following their instructions, we have written a review paper focused on the role of voltage-dependent potassium channels as oxygen-modulated ion channels in the carotid body. We have tried to make an up to date account of all the data available in the literature to provide an overview of our knowledge regarding the presence, the molecular and functional characterization and the role in oxygen chemotransduction in the carotid body of these channels.

This review is an original work that has not been published elsewhere, co-authored by Dr. J.R. López-López and myself.

Sincerely yours

Dr. M.T. Pérez-García

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Oxygen sensitive Kv channels in the carotid body

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Abstract

Hypoxic inhibition of K^+ channels has been documented in many native chemoreceptor cells, and is crucial to initiate reflexes directed to improve tissue O_2 supply. In the carotid body (CB) chemoreceptors, it is a general consensus regarding the facts that a decrease in pO_2 leads to membrane depolarization, increase of Ca^{2+} entry through voltage-dependent Ca^{2+} channels and Ca^{2+} -dependent release of neurotransmitters. Central to this pathway is the modulation by hypoxia of K^+ channels that triggers depolarization. However, the details of this process are still controversial, and even the molecular nature of these oxygen-sensitive K^+ (K_{O_2}) channels in the CB is hotly debated. Clearly there are inter-species differences, and even in the same preparation more than one K_{O_2} may be present. Here we recapitulate our present knowledge of the role of voltage dependent K^+ channels as K_{O_2} in the CB from different species, and their functional contribution to cell excitability in response to acute and chronic exposure to hypoxia.

Introduction

Aerobic organisms depend on oxygen for the production of energy and for biosynthetic reactions. Therefore, cells, tissues and organisms must be able to sense and respond to changes in the oxygen concentration of their environment. The response of mammalian cells to hypoxia is crucial for their survival, allowing cells to cope with a low oxygen environment. Among those responses to hypoxia, the ones of specialized cells located in chemosensory organs (carotid bodies, pulmonary neuroepithelial bodies, pulmonary artery smooth muscle among others) are unique as they differ both quantitative and qualitatively from the general self-defense reaction of the organism against low oxygen. The specific hypoxic response of these chemosensitive cells is rapid (seconds to minutes), has a lower threshold, shows little or no adaptation during the entire period of hypoxia, and associates with an increase in their metabolic activity leading to changes in their excitability, contractility or secretory activity. All these features are specializations directed to prevent hypoxia in the entire organism, and thus, to warrant homeostasis.

CB chemoreceptors participate in the ventilatory responses to acute and chronic hypoxia (CH). Arterial hypoxemia increases breathing within seconds, and CB chemoreceptors are the principal contributors to this reflex hyperventilatory response, as hypoxia induces release of neurotransmitters from CB chemoreceptor cells that increase the activity of the afferent chemosensory fibers (Gonzalez, C. et al. 1994). Central to the cardiorespiratory responses to hypoxia in most chemosensory tissues is the rapid inhibition of ion channels. In the CB, hypoxia depolarizes chemoreceptor cells by inhibiting K^+ channels, leading to the activation of voltage-dependent Ca^{2+} channels, Ca^{2+}

influx and exocytosis. Although this general mechanism seems to be present in all O₂-sensitive tissues (Youngson, C. et al. 1993; Weir, E. K. et al. 1995), there are inter-species differences in the molecular nature of the K_{O2}, and even in the same species more than one type of K_{O2} may be present (Peers, C. et al. 1995; Pérez-García, M. T. et al. 2000a; Patel, A. J. et al. 2001; Peers, C. et al. 2001).

Direct inhibition by hypoxia of a K⁺ channel was first demonstrated in chemoreceptor cells isolated from rabbit CB (Lopez-Barneo, J. et al. 1988) . Since then, many other K_{O2} have been found in several chemosensory preparations (Peers, C. 1997; Lopez-Barneo, J. et al. 2001; Patel, A. J. et al. 2001), and an intriguing aspect is the high degree of variability on the properties of these K_{O2} among different preparations, within the same preparation among different species, and within the same species among different developmental stages. Furthermore, there are evidences indicating that K_{O2} channels may be redundant, as more than one type of K_{O2} can coexist in the same preparation (Kemp, P.J. 2006), suggesting that the full hypoxic response of the cells may required the concerted action of more than one channel.

As a consequence of the high degree of diversity among K_{O2}, determination of the molecular identity of K_{O2} in native tissues as a previous step to explore the structural requirements for O₂ sensing in these channels has been and active field of research within the last years. Several K⁺ channel genes expressed in chemoreceptor cells have been identified, and some of them have been shown to behave as K_{O2} in heterologous expression systems. We have focused this review in those K_{O2} that produce voltage dependent K⁺ currents (Kv), since K_{O2} mediating background K⁺ currents or Ca²⁺ activated K⁺ currents are reviewed in other articles in this issue (Buckler ref; Peers ref). In the case of

Kv channels, the molecular identity of the native K_{O_2} has proven to be controversial. Since Kv channels are a family of proteins with a large molecular diversity (Coetzee, W. A. et al. 1999), it is a great challenge to assign the actual molecular identity to the native current observed in intact cells. The difficulty not only comes from the large number of candidate subunits, but also from the fact that heteromultimers formed between different subunits from the same family could give rise to currents with similar kinetics. The complexity is further enhanced by the fact that the association of auxiliary subunits and interacting proteins also changes channel properties. Yet, determination of the molecular constituents of the native K_{O_2} is an issue of capital importance to understand the molecular mechanisms of O_2 detection in chemoreceptor cells, to establish their physiological role in the native tissues, and additionally to provide a physiological meaning to the reported O_2 modulation of cloned channels expressed in heterologous systems (Hulme, J. T. et al. 1999; Perez-Garcia, M. T. et al. 1999; Conforti, L. et al. 2000).

Molecular diversity of Kv channels

K^+ channels are arranged in homo or heterotetramers of principal subunits (α), accompanied by evolutionarily unrelated auxiliary subunits. Principal subunits have been classified in several subfamilies according to their biochemical and electrophysiological features (reviewed by Coetzee, W. A. et al. 1999). They all share a common core of two transmembrane (TM) domains that flank a pore-forming (P) region (Figure 1A). The P-region shows a conserved signature (the sequence motif TxxTxGYGD) that accounts for the ionic selectivity filter of K^+ channels. The basic 2TM core forms the complete

subunit of some K⁺ channels, like the inward rectifiers subfamily (Kir) and the bacterial KcsA channels. However, the common core is complemented with a variable number of TM domains, giving rise to three different topological types of K⁺ channels (2P/4TM, 6TM and 7TM) present in metazoans (Figure 1A). The fourth TM segment (S4) of 6TM K⁺ channels contains a stretch with positively charged amino acids every third position and this is considered the key element for voltage sensing. 6TM are classified into several subfamilies (Kv, KCNQ, EAG, KcGMP, SK) according to their functional features (Hille, B. 2001).

The mammalian counterparts of the cloned Kv α subunits of *Drosophila* known as *Shaker*, *Shab*, *Shaw*, and *Shal* are designated Kv1, Kv2, Kv3, and Kv4, respectively. The Kv naming system was based on deduced phylogenetic relationships where channels that shared 65% sequence identity were assigned to a particular subfamily (Gutman, G. A. et al. 2003). At present, a parallel nomenclature designated KCN was developed by the Human Genome Organization to serve as a mechanism for classifying existing and newly discovered genes within the K⁺ channel superfamily. These four Kv channel subfamilies each consist of a number of members, including Kv1.1-1.9, Kv2.1-2.2, Kv3.1-3.4, and Kv4.1-4.3. In addition to this four subfamilies, there are other non-conducting α subunits, which include Kv5, Kv6, Kv8 and Kv9 subfamilies, that share greater sequence identity with Kv2 channels and can associate with them to modulate their function. Within a subfamily, a highly conserved T1 domain located in the hydrophilic amino-terminal region immediately preceding the S1 segment is the minimal requirement for subfamily-specific tetramerization of α -subunits. These T1 domains represent 'identity tags' that mediate subunit-subunit interactions for the Kv1 through Kv4

subfamilies and prevent heteromeric assembly of subunits belonging to different subfamilies.

Several K^+ channel auxiliary subunits have been identified that affect cell surface expression or modify specific functional properties of the pore-forming α subunits (Figure 1B). The first ancillary subunits described that associate with Kv channel α subunits were called Kv β subunits (Martens, J. R. et al. 1999; Biggin, P. C. et al. 2000). Members of the Kv β gene family are cytosolic proteins which interact with the NH₂-terminal domains of Kv1 α subunits or the COOH-terminal domains of some Kv2 and Kv4 α subunits, and serve numerous functions. They have been shown to increase current density by promoting α subunit surface expression early in biosynthesis, to alter the gating characteristics of some Kv α subunits by conferring rapid channel inactivation, and to invest with redox sensitivity to Kv α -Kv β channel complexes (Biggin, P. C. et al. 2000). Structurally, Kv β subunits couple in a 1:1 ratio with Kv α subunits and form a tetramer that docks tightly with the cytoplasmic N-terminal domain of Kv α subunits, the whole complex retaining a pseudo-fourfold symmetry.

The K^+ channel interacting protein (KChAP) is known to associate with Kv1, Kv2 and Kv4 family members to enhance cell surface expression without altering channel gating and thus it appears to function strictly as a chaperone (Kuryshhev, Y. A. et al. 2001). This is not the case for members of the KCNE gene family (minK, KCNE1 and MiRPs, KCNE2–4) which have been shown to associate with a KCNQ (Kv1.7) and erg channels, but also with some members of the Kv family, such as Kv3.4 and Kv4.2. The gene product is a small (129aa) single membrane-spanning protein that was originally named MinK (minimal K channel protein). Association of these KCNE proteins with the K channel α

proteins have been shown to have profound effects on channel gating and kinetics, drug sensitivity and voltage-dependent activation and inactivation (McCrossan, Z. A. et al. 2004). Also, for the Kv4 subfamily, several accessory subunits have been shown to co-immunoprecipitate and colocalize with Kv4 α subunits in heart and brain, indicating that they form stable complexes at the cell surface. Among those, three related proteins that specifically interact with Kv4 N-terminus were named K^+ channel interacting proteins 1–3 (KChIP1, KChIP2, KChIP3). KChIPs are cytoplasmic proteins that belong to the family of neuronal calcium sensor proteins, containing a conserved C-terminal core region and EF-hand domains (An, W. F. et al. 2000). The presence of the EF-hand domains suggests the possibility that KChIP association may confer Ca^{2+} regulation to the function of Kv4 channels. Functionally, KChIP are shown to be integral auxiliary subunits that alter the membrane presentation of Kv4 channels in a chaperone-like way and also modulate their gating properties. However, co-expression of Kv4 α subunits with KChIP does not fully recapitulate the kinetics of native currents. It has recently been shown that Kv4 channels also interact with certain dipeptidyl aminopeptidase (DPPX) proteins and that a complex involving DPPX, Kv4 α and KChIPs reconstitutes the properties of native Kv4 K^+ channels. Dipeptidyl aminopeptidases (DPP6 and DPP10, or DPPX and DDPY) are closely related to a family of non-classical serine proteases, but do not exhibit catalytic activity. DPP6 and DPP10 enhance Kv4.1 and Kv4.2 surface expression when co-expressed in oocytes and modulate the gating properties of these channels (Jerng, H. H. et al. 2004).

This brief overview of Kv channels structure provides some clues to understand the large functional heterogeneity of K currents. Their diversity

originates partly from the large number of genes that encode K⁺ channel α subunits, but also from alternative splicing, heteromeric assembly of pore-forming α subunits, RNA editing and post-transcriptional modifications. This multiplicity, displayed as a wide variability in gating kinetics, conductance, ion selectivity and pharmacology, is augmented by the interaction of K⁺ channels with their numerous ancillary proteins. In fact, in the light of recent findings ion channels can be envisioned as heteromeric, dynamically-assembled multiprotein complexes, to such extent that even though α subunits suffice to form a functional pore, it is not clear whether they actually do so in native tissues (Jerng, H. H. et al. 2004; McCrossan, Z. A. et al. 2004).

Kv channels and oxygen-sensitivity in the CB

The effect of oxygen tension modulating a voltage-gated outward K⁺ current in dissociated rabbit CB chemoreceptor cells (Lopez-Barneo, J. et al. 1988) propelled several groups to the electrophysiological characterization of this preparation, CB chemoreceptor cells. The results obtained are somewhat puzzling as they show important differences in the functional properties of CB chemoreceptor cells from different species and at different stages of development; besides, in spite of K_{O₂} being consistently found in all preparations, their electrophysiological and pharmacological profile also varies within species (see also Peers, *ref in this issue*, and Buckler, *ref in this issue*).

Kv channels in rabbit CB

In rabbit carotid body, a series of studies both in whole-cell and in excised patch preparations reveal the presence of three components in the whole-cell

K⁺ currents: a large-conductance Ca²⁺-activated current, a small conductance non-inactivating current and a small conductance fast inactivating current. Of these, only the latter K⁺ current is inhibited by hypoxia. Hypoxia inhibits this fast inactivating current (K_{O2} channels) in a reversible manner, by decreasing its open probability (Ganfornina, M. D. et al. 1992a). As the inhibition is also observed in excised membrane patches in the absence of intracellular mediators, a direct effect of hypoxia on K_{O2} or on a closely associated membrane protein was suggested (reviewed in Peers, C. 1997). More detailed kinetic and pharmacological characterization of Kv currents in rabbit carotid body have been provided by several studies (Ganfornina, M. D. et al. 1992b; López-López, J. R. et al. 1993). However, the minute size of the organ together with its structural complexity has delayed the attempts to establish the molecular identity of the currents by conventional molecular biology techniques. The use of dominant-negative constructs to probe the molecular nature of the K_{O2} represented a first step in that direction (Pérez-García, M. T. et al. 2000b). Dominant-negative constructs to block the expression of Kv1 (Kv1.xDN) and Kv4 (Kv4.xDN), as members of these two families contribute to transient outward currents in other preparations, were delivered into chemoreceptor cells by adenoviruses enabling ecdysone-inducible expression of the constructs. While infection of chemoreceptor cells with Kv1.xDN did not change the amplitude or the hypoxic sensitivity of K_{O2}, infections with Kv4.xDN suppressed transient Kv current in a time-dependent manner suggesting that native K_{O2} currents in rabbit CB are carried mainly by genes of the Kv4 subfamily. In addition, the observation that Kv4.xDN infection depolarizes CB chemoreceptor cells at rest and abolished hypoxia-induced depolarization suggest that Kv4

currents contribute to set resting membrane potential in this preparation. A more detailed molecular characterization of the expression of Kv α subunits that might underlie K_{O_2} currents demonstrated the expression in rabbit CB chemoreceptor cells of Kv3.4, Kv4.1 and Kv4.3 at the mRNA and protein level. Kv4.2 was absent and Kv1.4 was only found in sensory nerve endings (Sanchez, D. et al. 2002). Electrophysiological experiments applying specific toxins or antibodies demonstrate that both Kv3.4 and Kv4.x contribute to the fast inactivating K^+ current of chemoreceptor cells. However, while Kv4 subunits are homogeneously expressed in all chemoreceptor cells, Kv3.4 channels seem to be present only in a subset of cells. Furthermore, $[Ca^{2+}]_i$ measurements in dispersed CB cells under hypoxic conditions followed by immunocytochemical detection of Kv3.4 and Kv4.3 demonstrated that the presence of Kv3.4 in the chemoreceptor cell membrane is not required for the low PO_2 response. The results of this work lead to conclude that, due to their ubiquitous expression in chemoreceptor cells together with their electrophysiological properties, Kv4.1, Kv4.3, or their heteromultimers are the molecular correlate of the K_{O_2} currents in rabbit CB. Immunological blockade of K^+ channels to analyze the effect of antiKv3.4 or anti-Kv4.3 on the amplitude of the transient outward currents and their hypoxic sensitivity further supports this conclusion (Lopez-Lopez, J. R. et al. 2003). In patch-clamp experiments, both antibodies produced a significant reduction in the amplitude of the current demonstrating that these subunits are structural components of the channels that contribute to the fast inactivating K^+ current. After blocking Kv subunit-specific components, hypoxia could still decrease the remaining K^+ current in a significant manner. However, while anti-Kv3.4 antibody treatment does not modify in a significant way the magnitude of

hypoxic inhibition of K^+ current, in the presence of anti-Kv4.3 antibody this inhibition is significantly reduced, indicating that Kv4.3, but not Kv3.4 channels, contribute to the K_{O_2} currents. All these studies allow to conclude that Kv4 channels are the molecular correlate of K_{O_2} originally described in rabbit CB chemoreceptor cells.

However, several important questions regarding the role of these channels in the chemosensory response of the organ still remain. One question of paramount interest is to determine if hypoxic inhibition of these channels is the only trigger of the chemoreceptor signalling cascade. As mentioned above, the effect of hypoxia on rabbit CB chemoreceptor cells has been proposed to be mediated by a depolarizing shift in the resting membrane potential which leads to Ca^{2+} entry and neurotransmitter release. The activation threshold of Kv4 subunits (around -50 mV) makes them good candidates for controlling membrane potential, and data suggesting this role has been provide by the effect of adenoviral infection with the Kv4 dominant-negative (Pérez-García, M. T. et al. 2000b). Selective removal of the transient outward current is able to abolish the hypoxia-induced depolarization of the cells, and both hypoxia and 4-aminopyridine (1 mM) can induce depolarization of rabbit CB chemoreceptor cells. Although other targets for the effect of hypoxia on resting membrane potential can not be excluded, the fact that all these manoeuvres (AdKv4.xDN infection, low pO_2 and 1mM 4-AP application) are able to inhibit transient outward currents on the one hand and to depolarize CB chemoreceptor cells on the other, suggests a causal relationship between this two effects, and thereby a contribution of the Kv4-mediated transient outward current to set the normal resting membrane potential of rabbit CB chemoreceptor cells.

With respect to the possible role of Kv3.4, it is known that the presence of Kv3 channel subunits enables excitable cells to achieve fast repolarization of action potentials (reviewed by Rudy, B. et al. 2001), which in turn permits the repetitive firing of action potentials at high frequency. CB chemoreceptor cells are known to fire action potentials whose frequency increases with exposure to low PO₂ (López-López, J. R. et al. 1989). The expression of Kv3.4 could be involved in amplifying the hypoxic signal by increasing the firing frequency upon depolarization by low PO₂. In this scheme, an O₂-dependent blockade of the Kv3.4 should result in a prolonged spike repolarization, which in turn would decrease firing frequency. Thus, all the evidence gathered so far suggests that Kv3.4 would not be the O₂-sensitive channel component of the chemoreception cascade, but instead would play a modulatory role in chemoreceptor cell excitability. In fact, some recent reports implicate Kv3.4 channel expression in the adaptation of chemoreceptor cells responses in several physiopathological situations (see below).

Aside for these Kv channels, functional expression of other K⁺ channels have been reported in rabbit CB chemoreceptor cells, although the available data is scatter, and none of them have been demonstrated to participate in oxygen chemoreception. HERG-like currents have been shown to contribute to resting membrane potential, but their modulation by hypoxia has not been assessed (Overholt, J. L. et al. 2000). The expression of maxi-K currents has been consistently found in several preparations of rabbit CB chemoreceptor cells, using either electrophysiological or pharmacological tools. The amplitude of these currents exhibits a high cell to cell variability (Pérez-García, M. T. et al. 1992) and more importantly, they are not modulated by oxygen in this

preparation, which is in marked contrast with the proposed role of these channels in rat CB (see chapter by Peers in this issue). Finally, some pharmacological studies suggest the presence of ATP-dependent (K_{ATP}) and two-pore domain (TASK-like) K^+ channels, but there are not direct studies of these channels and no data regarding their role on hypoxic chemotransduction (Takahashi, T. et al. 2005).

Kv channels in rat CB

K_{O_2} currents have also been described in chemoreceptor cells from neonatal and adult rat CB. At least, there appear to be two distinct K^+ channels that respond to hypoxia thus causing depolarisation; firstly a high-conductance, Ca^{2+} -activated K^+ (maxiK or BK) channel (Peers, C. 1990; Pardal, R. et al. 2000; Riesco-Fagundo, A. M. et al. 2001) and secondly, an acid-sensitive tandem P domain K^+ channel, TASK (Buckler, K. J. et al. 2000). The properties of these two channels and their functional role in hypoxic signalling are described in detail in the chapters by Peers and Buckler in this issue. Although Kv currents are also present in this preparation, it is out of doubt that they do not represent the molecular correlate of K_{O_2} currents; instead, they may serve other physiological functions in the organ. In adult rat CB chemoreceptor cells, outward currents are carried mainly by K^+ , and two different components can be distinguished: A Ca^{2+} -dependent K^+ (maxiK) current sensitive to Cd^{2+} and charybdotoxin (ChTX) and reversibly inhibited by hypoxia, and a Ca^{2+} -insensitive, voltage-dependent K^+ current (IKv). IKv is blocked by TEA in the low mM range and is relatively insensitive to low concentrations of 4-AP. This pharmacological profile, together with its kinetic properties (slow voltage-dependent activation and very slow biexponential and voltage-independent

inactivation) define a classical delayed rectifier K^+ channel (López-López, J. R. et al. 1997), most likely of the Kv2 (*Shab*) subfamily, although no structural studies have been performed. The same two types of currents have been described in neonatal rat CB (Peers, C. et al. 1990). The relative contribution of these two components to fix resting membrane potential and consequently cell excitability is a key determinant of the altered response of the organ under physiological or physiopathological conditions. In this regard, changes on the relative expression of maxiK channels and IKv have been shown to correlate with the maturation of the response to hypoxia of the rat CB during development (Hatton, C. J. et al. 1997), and also provide an explanation to the sensitization of the response to acute hypoxic episodes after chronic hypoxia exposure in adult animals (Carpenter, E. et al. 1998) and to the blunting of the acute hypoxic responses in neonatal animals born in a chronically hypoxic environment (Wyatt, C. N. et al. 1995, see below). In this later situation, it has been shown that this blunting of the acute hypoxic response correlates with the decrease of functional maxiK channels and the lost of hypoxic-induced depolarization of chemoreceptor cells. However, the magnitude of hypoxic inhibition of K^+ currents was unchanged, indicating that acute hypoxia was modulating another K^+ current (IKv) that did not appear to influence resting membrane potential. As a corollary, it was suggested that chemoreceptor cells respond to hypoxia not because they possess specific K_{O_2} channels, but because they possess (as yet unidentified) specialized intracellular O_2 -sensing mechanisms, which can couple to different K^+ channel types, an interesting proposal that could also help to explain the large molecular diversity of K_{O_2} .

Kv channels in mouse CB

In the mouse CB initial studies have suggested important strain-dependent differences in the ventilatory responses to hypoxia, which seemed to have a correlate with differences in the structural and functional properties of the CB. Apart from the small size of the organ and its structural complexity and variability among strains, in this specie it has been found that the number of chemoreceptor is very low. Using a whole CB preparation (to preserve structural relationships) from DBA/2J strain (a hypoxia-responsive one) it has been shown the presence of outward K^+ currents that were reversibly inhibited by hypoxia and that could be classified in at least three different types: delayed rectifier, A-type and Ca^{2+} -activated K^+ currents (Yamaguchi, S. et al. 2004). In the presence of iberotoxin, hypoxia did not further inhibit K^+ currents, suggesting that K_{O2} currents in this strain are most likely through maxiK channels (Otsubo, T. et al. 2006). These authors proposed that differential expression of K^+ currents could contribute to differences in hypoxia sensitivity, and in fact maxiK mRNA (both the pore-forming α subunit and two different β subunits) seem to be more expressed in this DBA/2J strain than in A/J strain (a non-responsive one) although no quantification has been made.

A different approach to the characterization of the electrical properties of mouse CB chemoreceptor cells is the use of chemoreceptor cells in primary cultures obtained from a transgenic mouse expressing GFP under the control of tyrosine hydroxylase (TH) promoter (Sawamoto, Kazunobu et al. 2001). Immunocytochemical identification of TH in CB cell cultures reveals a good match with GFP positive cells (Perez-Garcia, M. T. et al. 2004). Furthermore, these cells show an increase in $[Ca^{2+}]_i$ in response to low pO_2 , demonstrating

their ability to engender a physiological response. Whole-cell experiments demonstrated slow-inactivating K^+ currents with a bi-exponential kinetic of deactivation. TEA sensitivity of the currents identified also two different components (IC_{50} of 17.8 ± 2.8 and $940.0 \pm 14.7 \mu M$). Current amplitude decreased reversibly in response to hypoxia, which selectively affected the fast deactivating component. Hypoxic inhibition was also abolished in the presence of low ($10\text{-}50 \mu M$) concentrations of TEA, suggesting that O_2 interacts with the component of the current most sensitive to TEA. Molecular biology and immunocytochemical techniques identify the expression of Kv2.2, Kv3.1, Kv3.2 and Kv3.3 subunits at the mRNA and for all but Kv2.2 also at protein level within single chemoreceptor cells. The pharmacological profile of the hypoxia sensitive component of Kv current makes Kv3 subunits prime candidates for the molecular correlate of the native K_{O_2} current.

Again, there are not studies on the functional contribution of these different K_{O_2} currents to chemoreceptor cells excitability in mouse CB. Further studies directed to explore the effect of hypoxia on resting membrane potential as well as the presence and possible O_2 modulation of other families of K channels (and their accessory subunits) will allow to determine the molecules involved in the hypoxic transduction cascade. However, this characterization will benefit of the possibility of using transgenic mice with deletion of some of the identified channels subunits as a tool to understand the contribution of these channels to excitability of the cells.

Kv channels in cat CB

In cultured cat CB cells, two types of cells were identified electrophysiologically, ones that had K_{O_2} and others whose K^+ currents were not

inhibited by hypoxia. However, these two types of cells were morphologically indistinguishable and the K^+ currents had similar pharmacological and kinetic properties (Chou, C. L. et al. 1996). They are non-inactivating outward K^+ currents sensitive to mM TEA. Combination of electrophysiological studies with immunocytochemical characterization demonstrated that only the cells positive for tyrosine-hydroxylase (TH) possessed K_{O_2} , and that only those cells were depolarized under hypoxic exposure (Chou, C. L. et al. 1998). However, no further studies conducted to study the functional contribution of K_{O_2} in these cells or their molecular correlates have been performed.

Oxygen-modulated Kv channels in other preparations

K_{O_2} currents are surprisingly ubiquitous, as they have been found in many tissues and cells (not only specialized oxygen-sensitive ones), and incredibly heterogeneous in terms of their molecular nature, not to talk about the mechanisms of O_2 regulation that is been covered in other chapters of this issue. Also in some of these preparations species-related differences in the nature of the K_{O_2} channels have been described.

In the case of **adrenal chromaffin cells**, it has been described that they possess a developmentally regulated O_2 -sensing mechanism, similar to carotid body type I cells. The work by Thompson and colleagues (Thompson, R. J. et al. 1997) shows that rat adrenomedullary chromaffin cells possess O_2 -sensing mechanisms, similar to their neural crest counterparts in the carotid body, and that these mechanisms are present in cells from neonatal animals but disappear during development. Electrophysiological experiments indicate that hypoxia is able to depolarize neonatal adrenal chromaffin cells and thus induce

neurosecretory response (Thompson, R. J. et al. 1997). Both in rat and in mouse cells, K_{O_2} currents included both Ca^{2+} -dependent (maxiK) and Ca^{2+} -independent, delayed rectifier type (IKv) (Thompson, R. J. et al. 1998; Thompson, R. J. et al. 2002). However, at least in the rat adrenal chromaffin cells, hypoxic inhibition of these two types of channels does not seem to account for cell depolarization, as this depolarization was unchanged in the presence of selective blockers of maxiK channels and was not fully prevented after blockade of IKv with TEA and 4-aminopyridines, suggesting the existence of additional O_2 -sensing mechanism that controls at least part of the initial depolarization or receptor potential during anoxia and that is insensitive to conventional blockers of Kv channels (Thompson, R. J. et al. 1998).

In **neuroepithelial body (NEB) cells** of foetal rabbit and neonatal human lungs the H_2O_2 -sensitive channel Kv3.3a has been shown to be co-expressed with the membrane components of the NADPH oxidase (gp91^{phox} and p22^{phox}), and to respond to hypoxia with a reversible inhibition (Wang, D. et al. 1996). Although it has been reported that hypoxia fails to inhibit NEB K^+ channels in gp91^{phox} knock-out mice (Fu, X. W. et al. 2000), NADPH oxidase is, however, not a universal oxygen sensor, as hypoxic inhibition of K^+ channels in pulmonary artery smooth muscle cells is unaltered in oxidase knock-out animals (Patel, A. J. et al. 2001). Electrophysiological studies of “intact” NEB performed in fresh neonatal rabbit lung slices indicate that outward K^+ currents could be dissected into two components, a Ca^{2+} -dependent and a Ca^{2+} -independent, being both components roughly equally suppressed by hypoxia. A similar pattern of expression of K_{O_2} currents have been also found in mouse NEB in lung slices (Fu, X. W. et al. 2000).

In **pulmonary artery smooth muscle cells** (PASMC), based on what we know so far, Kv channels appear to be top candidates as the K_{O_2} channels, although other types of K^+ and Ca^{2+} channels are also regulated by hypoxia (reviewed in Patel, A. J. et al. 2001; Yuan, J. X. 2001). The summary of the work of several laboratories, using different approaches, has provide compelling evidence indicating that Kv2.1, Kv1.2, Kv1.5 and Kv3.1b, some of them probably as heteromultimers, may encode K_{O_2} channels in PASMC. The finding of differential expression of several Kv channel auxiliary proteins such as Kv β subunits and Kv9.3 subunits provide a new concept by strongly suggesting that these accessory subunits of the pore-forming Kv α subunit as well as their heterologous assembly with different α subunits may play a critical role in determining the oxygen sensitivity of Kv channels in PASMC (Patel, A. J. et al. 2001; Yuan, J. X. 2001). The study of whether these subunits form the native Kv channels that are dominantly involved in the regulation of resting membrane potential and which channel subunit combinations have a physiological role in determining oxygen-sensitivity of PASMC constitute fields of intense research. However, in the last years is becoming more evident that there will not be only one K_{O_2} channel in PASMC. Rather, the oxygen sensor responsible for hypoxic pulmonary vasoconstriction would be better defined as an oxygen-sensing system in which multiple "oxygen sensors" coordinate with each other and transduce hypoxic signals to different "effectors" (i.e., the different Kv channel α and β subunit combinations) to ensure efficacy (Yuan, J. X. 2001).

Finally, it is noteworthy to point out that a number of studies have been focused to determine the sensitivity to hypoxia of recombinant Kv channel in **heterologous expression systems**, including *Xenopus* oocytes, mouse L-cell

line, CHO cells, rat adrenal PC12 cells, COS-7 cells, and HEK-293 cells (reviewed in Lopez-Barneo, J. et al. 2001; Patel, A. J. et al. 2001; Yuan, J. X. 2001). Potential candidates to form K_{O_2} channels include Kv1.2, Kv1.5, Kv1.2/Kv1.5 Kv2.1 Kv2.1/Kv9.3 Kv3.1b, Kv3.3 and Kv4.2/Kv β 1.2. In all cases, acute hypoxia significantly reduces the K^+ currents generated by these channels, and their hypoxic sensitivity appears to be independent of the expression systems. These experiments with recombinant channels open the way to investigate hypoxic inhibition at the molecular level, providing important information to understand the molecular basis of oxygen-sensitivity by ion channels, as they contribute to resolve an issue of paramount importance, that is whether the channels can themselves directly act as O_2 sensors.

Role of carotid body Kv channels in chronic hypoxia adaptation

Prolonged periods of hypoxia, either the ones experienced by healthy individuals at high altitude or as a consequence of cardiorespiratory disorders, result in significant cellular adaptation or remodelling, leading to changes in cellular excitability. Altered ion channel expression by chronic hypoxia (CH) is an important component of this functional adaptation, contributing directly to the modified tissue response to hypoxia. The mechanisms by which CH, as opposed to acute hypoxia, triggers altered functional expression of ion channels are of cardinal interest from a physiological point of view, since they probably contribute to adaptation to high altitude, but also and more importantly from a physiopathological perspective, as damage in certain respiratory and cardiovascular diseases is to a large extent secondary to prolonged exposure to low O_2 levels.

The ventilatory acclimatization to hypoxia is almost exclusively dependent on CB (Bisgard, G. E. 2000) where CH exposure induces profound morphological and neurochemical changes (Wang, Z. Y. et al. 2002) together with increased chemosensitivity. Reports concerning the effects of prolonged hypoxia on the acute hypoxic sensitivity of the CB are mixed, and some discrepancies might be accounted for by species-dependent rates of adaptation of the CB to CH. Long term exposure to hypoxia leads to an enhancement of acute hypoxic reactivity that within some time (weeks to years, depending on species) changes into a blunting of the response to acute hypoxia. As the chemotransduction cascade begins with the inhibition of K_{O_2} , it has been hypothesized that changes in the expression levels or in the O_2 sensitivity of K_{O_2} could contribute CH adaptations. In fact, in PC12 cells the effect of CH on the expression pattern of K^+ currents has been studied, and up-regulation of the K_{O_2} current (Kv1.2) has been proposed as the mechanism of hypoxic sensitisation (Conrad, P. W. et al. 2001). Similarly, the changes in the expression of the O_2 -sensitive components of the K^+ current has been implicated in the loss of hypoxic sensitivity of pulmonary artery smooth muscle cells upon exposure to prolonged hypoxia (Platoshyn, O. et al. 2001; Pozeg, Z.I. et al. 2003). In the rat CB, blunting of the acute hypoxic response in animals born and raised under CH has been demonstrated to correlate with the down-regulation of the K_{O_2} current (maxiK channels, (Wyatt, C. N. et al. 1995). On contrast, in the rabbit CB chemoreceptor cells a different mechanism that results in modification of hypoxic sensitivity by CH without affecting the expression of K_{O_2} channels has been described. CH *in vitro* is able to induce changes in the expression profile of Kv channels of chemoreceptor cells that

lead to an increase of hypoxic sensitivity of K^+ currents (Kaab, S. et al. 2005). Exposure of CB primary cultures during 24-72 h to low levels of O_2 (5%; ~33 mmHg) produces a specific down-regulation of Kv3.4 channel (both at the protein and mRNA levels) that accounts for the decrease in the fast transient outward K^+ current that can be observed in the whole-cell experiments. Importantly, as the K_{O_2} current in this preparation has been identified as belonging to the Kv4 family, down-regulation of Kv3.4 gene leads to a decrease of the O_2 -insensitive component of the K^+ current, resulting in a more predominant role of K_{O_2} in the total outward current, with the subsequent relative increase in the magnitude of acute low PO_2 -induced inhibition. Moreover, under CH conditions, acute hypoxia induced a significantly larger depolarization of resting membrane potential and consequently increased excitability, indicating that this ionic remodelling could explain CH sensitization. These results imply that in rabbit CB chemoreceptor cells acute and chronic hypoxia are both able to modulate ion channels, but exert their effects acting through different molecules: while acute exposure to hypoxia decreases the activity of Kv4 channels, prolonged hypoxia down-regulates Kv3.4 channels, and doing so increases the relative contribution of Kv4 channels to the total outward current (Figure 3). The data of Peers' group in rat CB (Carpenter, E. et al. 1998) could be reinterpreted in a similar fashion, as they show that CH decreases K^+ current density because of a reduction of the Ca^{2+} -insensitive K^+ current (IKv) component without changes in the maxiK current amplitude. Their results also indicate that adult rat CB chemoreceptor cells undergo a selective suppression of Ca^{2+} -insensitive, voltage-gated K^+ currents in response to CH in

vivo, which results on an increased proportion of the K_{O_2} current to the total currents.

Whilst this mechanism may participate in adaptive responses such as high-altitude acclimatization, such altered channel expression may also contribute to the onset of pathological disorders. In this regard, the same functional changes and the same remodelling of Kv channels (a decrease of Kv3.4 channels without changes in Kv4 expression) described under CH in rabbit CB (Kaab, S. et al. 2005) has been reported in rabbit CB from a model of congestive heart failure (Li, Yu Long et al. 2006). Chronically impaired cardiac output in this animal model might be sufficient to render a prolonged deprivation of oxygen delivery to chemoreceptor cells, a state akin to CH, leading to activation of renin-angiotensin system in the CB. The up-regulation by CH of this local angiotensin system contributes to altered sensitivity of CB to acute hypoxia, as angiotensin II increases the sensitivity of Kv channels to hypoxia and the hypoxia-induced depolarization (Li, Yu Long et al. 2006).

In general, while acute hypoxic responses occur on a time scale of seconds or less and involve the modification of pre-existing proteins, CH responses occur on a time scale of minutes or more and involve changes in gene expression leading to new protein synthesis. The discovery of the transcription factor HIF-1 has led to the delineation of molecular mechanisms of O_2 -regulated gene expression that are operative in all cells including those within the carotid body (see Lahiri, S. et al. 2006). Although HIF-1 mediated regulation of ion channels under CH have been shown in pulmonary artery smooth muscle cells (Aaronson, Philip I. 2006), data regarding its role in CB is still awaiting further experimentation.

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Figure Legends

Figure 1. **A.** Phylogenetic unrooted tree derived from maximum likelihood analysis of the amino acid sequences of the region encompassing the S5-P-S6 domains of representative examples of human K channel subunits. Local bootstrap proportions values above 50% are indicated in each branch. The scale bar represents branch length (number of amino acid substitutions/100 residues). **B.** Molecular composition of Kv channels. Kv channels are heterotetramers of principal (α) subunits and several auxiliary subunits that affect cell surface expression or modify specific functional properties of the pore-forming α subunits. The scheme shows the location (transmembrane or intracellular) of the Kv channels auxiliary subunits and their proposed binding site in the Kv α subunit.

Figure 2. Suppression of Kv4 channels modifies rabbit CB chemoreceptor cells excitability. Current-clamp experiments using the perforated patch technique were performed in control cells and in Kv4.xDN infected cells. The left part of the figure shows the values of resting membrane potential in a control cell during the application of a hypoxic solution and a solution containing 1mM 4-AP. Simultaneous register of the PO₂ value in the bath solution is shown in the lower part of the graph. The average depolarization obtained with these two manoeuvres is represented in the inset as ΔV_m (** $p < 0.001$). The effect of hypoxia was studied using the same experimental protocol in a Kv4.xDN infected cell (right panel). Resting membrane potential values were more depolarized in these cells. In addition, hypoxia did not produce any significant change on the resting membrane potential in all the Kv4.xDN studied, as shown in the inset. (Modified from Perez-Garcia, M. T. et al. 2000).

Figure 3. Scheme of the proposed model for the effects of acute and chronic hypoxia on rabbit CB chemoreceptor cells. Kv4 channels are the targets of acute hypoxic inhibition, leading to cell depolarization, opening of voltage-dependent Ca^{2+} channels and increased neurotransmitter release. Under prolonged (chronic) hypoxic exposure, a decreased expression of Kv3.4 channels through a yet undetermined pathway takes place. This down-regulation of Kv3.4 channels determines a larger contribution of Kv4 channels to the total outward current that could explain the sensitization of the response of chemoreceptor cells to acute hypoxic episodes under these conditions (see text for more details).