Experimental Physiology

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Hypoxic regulation of ion channel function and expression

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Acute hypoxia regulates the activity of specific ion channels in a rapid and reversible manner. Such effects underlie appropriate cellular responses to hypoxia which are designed to initiate cardiorespiratory reflexes and contribute importantly to other tissue responses, all of which are designed to improve tissue O_2 supply. These responses include excitation of chemoreceptors as well as pulmonary vasoconstriction and systemic vasodilatation. However, such responses may also contribute to the adverse responses to hypoxia, such as excitotoxicity in the central nervous system. Whilst numerous ion channel types are known to be modulated by acute hypoxia, the nature of the O_2 sensor in most tissues remains to be identified. Prolonged (chronic) hypoxia regulates functional expression of ion channels, and so remodels excitability of various cell types. Whilst this may contribute to adaptive responses such as high-altitude acclimatization, such altered channel expression may also contribute to the onset of pathological disorders, including Alzheimer's disease. Indeed, evidence is emerging that production of pathological peptides associated with Alzheimer's disease is increased during prolonged hypoxia. Such effects may account for the known increased incidence of this disease in patients who have previously endured hypoxic episodes, such as congestive heart failure and stroke. Identification of the mechanisms coupling hypoxia to the increased production of these peptides is likely to be of therapeutic benefit. *Experimental Physiology* (2002) **87.4**, 413–422.

Contents Page

Oxygen sensing by the carotid body

The mammalian respiratory and cardiovascular systems have developed to optimize the collection of O_2 from the environment and to deliver this O_2 to respiring tissues in an efficient manner. O_2 is necessary for the mitochondrial generation of ATP, and so for providing energy to fuel numerous cellular reactions essential to survival. It is, therefore, perhaps unsurprising that mechanisms have developed to monitor these important processes of O_2 collection and delivery, and to initiate rapid responses when these processes are compromised. The ability to sense changes in local O_2 levels permits appropriate cellular and tissue responses that can improve O_2 collection in the lungs and optimize its appropriate delivery according to the varying demands of numerous tissue types around the body. This role of monitoring O_2 levels is served in part by specific chemoreceptors.

The carotid bodies are the major arterial chemoreceptors (Gonzalez *et al.* 1992, 1994). They are tiny organs that sense O_2 levels (as well as CO_2 levels and pH) in arterial blood (Fig. 1*A*). For decades people have understood that when arterial blood becomes hypoxic, the carotid body responds by increasing the discharge levels of afferent chemosensory fibres in the carotid sinus nerve (Gonzalez *et al.* 1994). In this way information concerning blood gas content is rapidly relayed to the respiratory centre of the brain, and reflex changes in breathing patterns (as well as complex cardiovascular responses) are initiated. Despite

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this long-standing knowledge, the mechanisms by which the carotid body senses O_2 levels remained unknown, although indirect evidence pointed to type I (glomus) cells within the organ as being of major importance (Fig. 1*B*). These cells lie in close, synaptic contact with afferent chemosensory fibre terminals, and they are filled with a variety of neurotransmitter substances. Release of such transmitters (when studied by radiolabelling vesicular transmitter stores within intact carotid bodies) appeared to correlate with increased afferent nerve activity during hypoxia (see e.g. Obeso *et al.* 1992, and references therein), suggesting that transmitter release from type I cells was a key step in carotid body chemotransduction. These findings led to the development of isolated type I cell preparations so that, cellular physiological techniques could be applied in order to study hypoxic stimulus–secretion coupling for the first time.

Oxygen-sensitive K^+ channels

A major breakthrough in understanding the cellular basis of carotid body chemoreception came in 1988, when the first report indicating that hypoxia could regulate the activity of specific ion channels in isolated type I cells was published (Lopez-Barneo *et al.* 1988). In this study, K^+ channels of the rabbit carotid body were shown to be inhibited reversibly by hypoxia. This report, and others which followed soon after from other groups (Hescheler *et al.* 1989; Peers, 1990; Stea & Nurse, 1991; and e.g. Fig. 1*C*), initiated the development of the membrane hypothesis for hypoxic chemoreception. Put simply, this hypothesis suggested that hypoxic inhibition of K^+ channels caused membrane depolarization (e.g. Fig. 1*D*) or increased excitability, leading to increased Ca^{2+} influx via voltage-gated Ca^{2+} channels and hence neurotransmitter release. The details of this response varied between species studied (especially concerning the identity of the specific K^+ channels involved; Peers & Buckler, 1995) but the concept that ion channels could be regulated in a rapid manner by local O_2 levels was born.

Further studies in rabbit type I cells characterized the O_2-sensitive K^+ channel as being a voltage-gated, inactivating

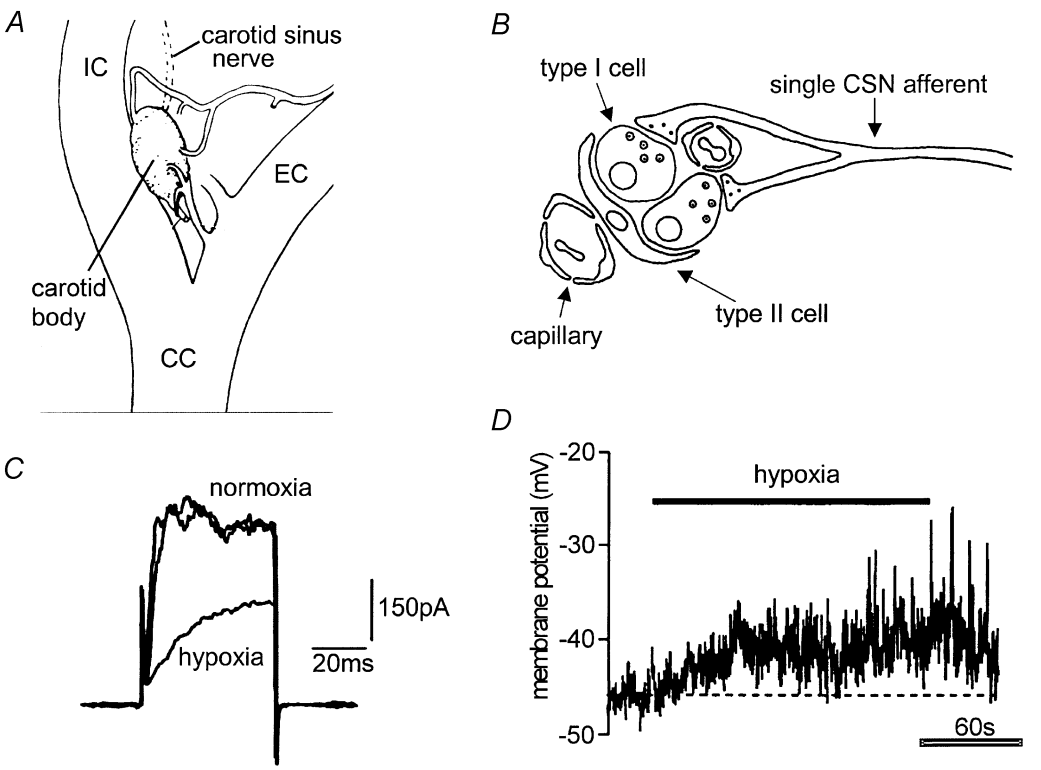

Figure 1

A, drawing of the carotid body chemoreceptor, which is located at the bifurcation of the common carotid artery (CC) into the internal (IC) and external (EC) branches. *B*, drawing of the cellular arrangement of the carotid body. Afferent chemosensory fibres terminate and are in synaptic contact with transmitter-filled type I (or glomus) cells. Clusters of type I cells are encapsulated by type II cells, and the organ receives a rich supply of arterial blood. *C*, outward K^+ currents recorded in a voltage-clamped, isolated type I cell using the whole-cell patch-clamp technique. Currents were evoked by step depolarizations to +20 mV from a holding potential of –70 mV. Note reversible current inhibition by hypoxia. *D*, membrane potential monitored in another type I cell using a current-clamp, perforated patch recording. Note that hypoxia causes membrane depolarization. This effect is modest, but is sufficient to activate voltage-gated Ca^{2+} channels that are present in these cells. Reproduced from Wyatt & Peers (1995).

K^+ channel, and regulation by hypoxia was highly selective, since two other K^+ channel types also present in these cells were shown to be O_2-insensitive (Ganfornina & Lopez-Barneo, 1991). Whilst these findings clearly characterized this channel, studies by others indicated that there was species variation in the nature of the K^+ channel suppressed by hypoxia in the carotid body. In the rat, the O_2-sensitive K^+ channel was first identified as a high conductance Ca^{2+}-activated K^+ channel (maxi-K channel; Peers, 1990). Inhibition of these channels either by hypoxia or specific peptide toxins caused membrane depolarization (Wyatt *et al.* 1995; Wyatt & Peers, 1995), and more recently, this inhibition has been shown to cause dopamine release in a novel carotid body slice preparation (Pardal *et al.* 2000). However, this type of channel seems ill-suited to the role it appears to serve in the carotid body, since the rise of $[Ca^{2+}]_i$ caused by depolarization-evoked opening of voltage-gated Ca^{2+} channels during hypoxia (caused by hypoxic inhibition of K^+ channels) would be expected to re-activate these channels, and so make the response of the cell transient. Clearly, the response of the carotid body to hypoxia is a sustained one. In order to understand this apparent discrepancy, we have recently studied the interactions of hypoxia and Ca^{2+} on maxi-K channels, using a HEK 293 cell stably transfected with both α and β subunits of the channel cloned from human brain. These channels are also inhibited by hypoxia when they are activated by both Ca^{2+} and voltage. However, in the virtual absence of Ca^{2+} these channels can still be activated by voltage alone, and under such conditions hypoxia is without effect on channel activity (Lewis *et al.* 2002). Thus, hypoxia appears to interfere with Ca^{2+} activation of these channels. If this is also the case for maxi-K channels in the carotid body, it is likely that channel activity could remain suppressed despite a rise of $[Ca^{2+}]_i$.

In 1997, Buckler described the activity of another O_2-sensitive K^+ conductance in rat type I cells (Buckler, 1997). This paper turned out to be the first report of the activity of a native member of a brand new family of K^+ channels, the so-called 'leak', 'background' or tandem-P domain channels (reviewed by Goldstein *et al.* 2001). These channels are unique in many ways, but primarily because of their structure: each subunit possesses two P-domains (each of which forms one-quarter of the pore lining of a K^+ channel), and forms functional channels as dimers. In the carotid body, the particular channel was identified as TASK-1, an acid-sensitive member of this family (Buckler *et al.* 2000). These channels are of low conductance and are voltage insensitive – ideal properties for influencing membrane potential. It remains to be resolved which of these two channels (maxi-K or TASK-1) are the most important in controlling the excitability of type I cells, and at present the available evidence suggests that both contribute significantly to this important role.

Shortly following the reports that carotid body K^+ channels could be rapidly regulated by changes in O_2 levels, evidence emerged that similarly regulated K^+ channels existed in other tissues, including airway chemoreceptors (neuroepithelial bodies; Youngson *et al.* 1993), smooth muscle cells of the pulmonary vasculature (Post *et al.* 1992), central neurons (Jiang & Haddad, 1994) and cardiac myocytes (Hool, 2001). A comprehensive list of O_2-sensitive channels can be found elsewhere in excellent recent reviews (Patel & Honore, 2001; Lopez-Barneo *et al.* 2001). What is clear from these studies is that such channels are not confined to chemoreceptor cells. Furthermore, local O_2 levels can regulate numerous different types of K^+ channel. The phenomenon of O_2-sensitive K^+ channels is clearly widespread, and likely to grow further. One recent example comes from studies of K^+ channel activity in primary cultures of cerebellar granule neurons. These cells express TASK-1 channels that can be rapidly and reversibly inhibited by acute hypoxia, resulting in membrane depolarization and increased input resistance (Plant *et al.* 2002). The important influence of these channels on excitability in these neurons suggests that their inhibition by hypoxia may contribute to hyperexcitability and perhaps excitotoxicity during hypoxic/ischaemic conditions.

Oxygen-sensitive Ca^{2+} channels

In 1995, Lopez-Barneo and colleagues demonstrated that L-type Ca^{2+} channels recorded from isolated smooth muscle cells of various systemic vascular beds could be reversibly inhibited by hypoxia (Franco-Obregon *et al.* 1995). This was the first report of a channel other than a K^+ channel being O_2 sensitive. Inhibition was strongly voltage dependent, and appeared to be associated with a slight slowing of activation. This fundamental observation is potentially extremely important, since systemic vascular tone is dependent on Ca^{2+} influx through L-type channels, and their inhibition by hypoxia could go a long way towards explaining systemic hypoxic vasodilatation. L-type Ca^{2+} channels, like most other voltage-gated Ca^{2+} channels, are heteromultimeric proteins, consisting of a major, pore-forming and voltage-sensing α_1 subunit (see Fig. 2) which is associated with auxiliary β and α_2-δ subunits (and, at least in some cases, an additional γ subunit; Catterall, 1995). Following the study of Franco-Obregon *et al.* (1995), we examined the effects of hypoxia on human α_{1C} subunits expressed alone in HEK 293 cells. This channel subunit was originally cloned from human cardiac tissue (Schultz *et al.* 1993), although it is also present in vascular smooth muscle (Klockner *et al.* 1997). Despite the lack of auxiliary subunits, currents generated by expression of α_{1C} subunits responded to hypoxia in a manner indistinguishable from the effects seen on native L-type Ca^{2+} channels of vascular smooth muscle (Fearon *et al.* 1997). This report was the first to demonstrate hypoxic inhibition of a recombinant ion channel, and further indicated that O_2 sensing was a property of the channel that did not require auxiliary subunits. In addition, the relationship between degree of hypoxia and level of inhibition indicated that these channels were tonically regulated over a wide range of physiologically relevant P_{O_2} levels (Fearon *et al.* 1997).

The human α_{1C} subunit exists in three different forms, arising from splice variation that occurs in a restricted region of the intracellular C-terminal tail (Fig. 2). When

first cloned and expressed in a recombinant system, these three variants appeared functionally identical (Klockner *et al.* 1997), and so the physiological reason for such splicing remained undetermined. However, we found them to be differentially regulated by hypoxia. In fact, only one variant, containing a 71 amino acid insert, displayed any O_2 sensitivity at all (Fearon *et al.* 2000). This immediately indicated that this region of the channel was both necessary and sufficient for O_2 sensing. Subsequent molecular manipulations confirmed this, and restricted the region to a 37 amino acid region. To date, these findings represent the most detailed knowledge of the structural requirements for O_2 sensing of any ion channel. Whether or not this region of the protein confers O_2 sensitivity on these channels alone, or contains a structural signature common to other O_2-sensitive channels, remains to be determined.

The nature of the oxygen sensor

Despite these advances in our understanding of acute O_2 sensing by ion channels, the nature of the O_2 sensor itself remains a mystery. Although it is clear from many of the studies described above that channels known to be O_2 sensitive in certain cell types retain the ability to respond to hypoxia in recombinant expression systems, some key observations suggest strongly that O_2-sensitive channels themselves are not the actual sensors. Firstly, Lopez-Lopez & Gonzalez (1992) demonstrated elegantly that hypoxic inhibition of K^+ channels in rabbit type I carotid body cells could be reversed by carbon monoxide, a finding which strongly suggested the involvement of a haem moiety. Such moieties are not recognized as forming part of a channel. Secondly, Patel *et al.* (1997) cloned an O_2-sensitive K^+ channel from pulmonary smooth muscle, yet when it was

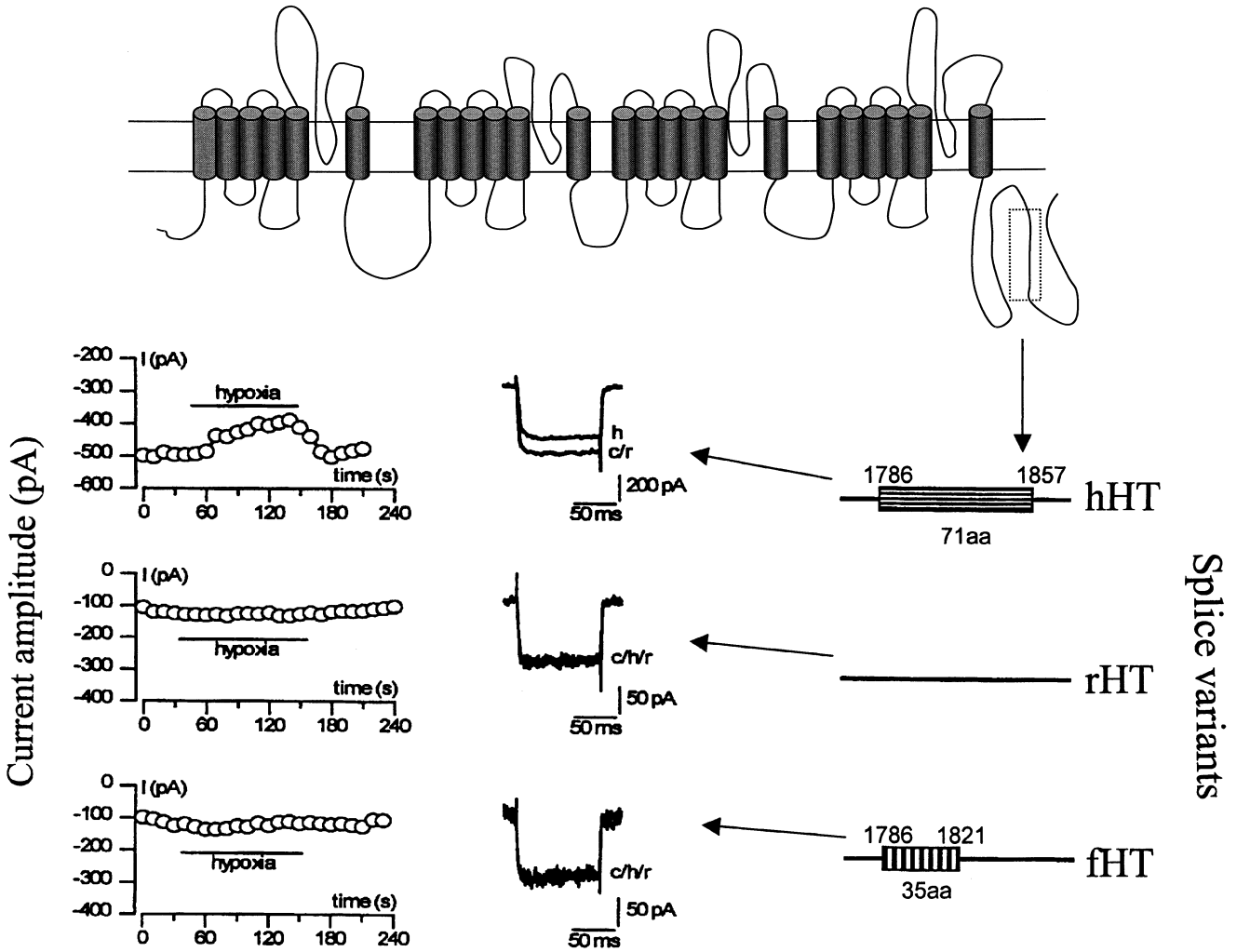

Figure 2

Upper trace shows a drawing of the structure of the α_{1C} subunit of a voltage-gated, L-type Ca^{2+} channel. The dashed box indicates the region of the channel protein that undergoes splice variation. The schematic diagram (below, right-hand side) indicates the three channel variants arising from splicing. The traces and plots shown on the left-hand side indicate the differential responses of these splice variants to acute hypoxia when the channels are expressed in HEK 293 cells. Note that only the upper variant (containing a 71 amino acid insert) displays O_2 sensitivity. Modified from Fearon *et al.* (2000).

expressed in COS cells, hypoxic inhibition was only demonstrable in a sub-population of cells, whilst all other features of the channel were consistently reproduced. This suggested heterogeneous distribution of an additional factor (or sensor) in this recombinant expression system.

To date, the only convincing candidate O_2 sensor is NADPH oxidase, a membrane-associated enzyme complex that uses NADPH as an electron donor to generate superoxide from molecular oxygen. It is believed that the superoxide is then rapidly dismutated to hydrogen peroxide, which in turn controls channel activity via redox modulation. However, this has only been established for one particular cell type, the airway chemoreceptor, or neuroepithelial body (Fu *et al.* 2000). The involvement of NADPH oxidase in the regulation of K^+ channels in these cells was first suggested following the finding of the ability of the oxidase inhibitor, diphenylene iodide, to prevent hypoxic channel inhibition (Youngson *et al.* 1993). Despite the fact that this agent can block certain K^+ channels directly (Weir *et al.* 1994), further support came from small cell lung carcinoma (H146) cells, which are believed to be immortalized neuroepithelial body cells. In H146 cells, two distinct oxidase inhibitors suppressed hypoxic inhibition of O_2-sensitive K^+ channels (O'Kelly *et al.* 2001), and activation of protein kinase C suppressed hypoxic inhibition by stimulating the oxidase to generate superoxide even during hypoxic conditions (O'Kelly *et al.* 2000). However, the clearest evidence that the oxidase acts as an O_2 sensor, comes from studies in a knockout mouse model that lacks the major oxidase enzyme subunit, $gp91^{phox}$. In these mice, hypoxic inhibition of neuroepithelial K^+ channels was completely abolished (Fu *et al.* 2000). However, it is important to note that O_2 sensing by other tissues (the carotid body, He *et al.* 2002; and pulmonary vasculature, Archer *et al.* 1999) was unaffected in this knockout model. This clearly indicates that other O_2-sensing mechanisms predominate in other tissues, and suggests that NADPH oxidase only acts as an O_2 sensor coupled to K^+ channel activity in airway chemoreceptors.

The nature of the O_2 sensor(s) coupled to regulation of ion channels thus remains to be identified in most cell types. Perhaps the most convincing evidence is that provided by Lopez-Lopez & Gonzalez (1992), described above, suggesting the presence of a haem-containing molecule based on the reversibility of hypoxic inhibition by carbon monoxide. Indeed, the same workers have further supported this idea by showing similar modulation of maxi-K channels in rat type I cells (Riesco-Fagundo *et al.* 2001), and carbon monoxide regulation of recombinant voltage-gated K^+ channels (Perez-Garcia *et al.* 1999). It is also noteworthy that mitochondrial inhibitors can mimic at least some of the effects of hypoxia in other O_2-sensing tissues, including the carotid body (Buckler & Vaughan-Jones, 1998), supporting a long-standing suggestion that these organelles can indeed act as O_2 sensors. Furthermore, mitochondria are presently taking centre stage in the pulmonary vasculature, the vascular bed that uniquely responds to hypoxia by constricting in order to divert blood to better-ventilated regions of the lung (Waypa *et al.* 2001; Leach *et al.* 2001). Clearly, this is a fundamental issue requiring further investigation in order to understand acute O_2 sensing at the molecular level.

Remodelling of ion channel expression by chronic hypoxia

Prolonged periods of hypoxia are experienced by healthy individuals at high altitude, and also as a result of numerous cardiorespiratory disorders including chronic obstructive pulmonary disease and congestive heart failure. Such chronically hypoxic conditions can lead to profound changes in the properties of several different tissue types, such as hypertension in the pulmonary circulation and altered carotid body chemosensation. Available evidence suggests that altered functional expression (or remodelling) of ion channels was associated with such effects, and probably accounted for the altered responsiveness of such tissues. Thus, Nurse and colleagues reported that *in vitro* culturing of type I carotid body cells under hypoxic conditions led to a selective up-regulation of tetrodotoxin-sensitive Na^+ channels, an effect that could be mimicked by (and thus probably involved) exposure to raised cAMP levels (Stea *et al.* 1992). Such an effect rendered type I cells more electrically excitable, and could therefore contribute to the increased sensitivity of the carotid body to acute hypoxia during hypoxic acclimatization.

Effects of *in vivo* chronic hypoxia have been studied in both the pulmonary vasculature and the carotid body. Smirnov *et al.* (1994) demonstrated that isolated pulmonary smooth muscle cells of rats maintained in a hypoxic environment were depolarized as compared with cells from rats maintained in normoxic conditions, due to the selective down-regulation of a delayed rectifier K^+ current. Such an effect is likely to be a major contributory factor in pulmonary hypertension associated with hypoxic adaptation. In the carotid body type I cell, maxi-K channel functional expression was suppressed in rats born and reared in a hypoxic environment as compared with controls (Wyatt *et al.* 1995). These hypoxic cells failed to depolarize in response to acute hypoxia, an observation that is in keeping with the reduced acute hypoxic sensitivity of ventilation in these animals. Indeed, the properties of type I cells from hypoxic animals were similar to the properties of immature animals born and kept in a normoxic environment (i.e. lowered functional expression of maxi-K channels; Hatton *et al.* 1997), suggesting that maintenance of animals in hypoxic conditions from birth suppresses the postnatal maturation of this tissue by inhibiting the expression of specific cell types.

Altered expression of ion channels has been documented in a variety of other tissue types, including O_2-sensitive cell lines (Conforti & Millhorn, 1997; Green & Peers, 2001), which are much more amenable to investigation of the underlying molecular mechanisms. At present, this field is in its infancy and much further work is required to examine the question of how prolonged hypoxia (as opposed to acute hypoxia) triggers altered functional expression of ion

channels. This question is of physiological interest, since it probably contributes to the adaptations to high altitude conditions. However, perhaps more important is the question of the involvement of prolonged hypoxia in certain disease states, since it may contribute to more far-reaching detrimental conditions which are secondary to lowered O_2 levels.

Chronic hypoxia and its relationship to dementia

Numerous cardiorespiratory disease states (e.g. chronic obstructive pulmonary disease or congestive heart failure) result in a persistent lowering of arterial O_2 levels. It is important to note that these conditions can lead to impairment of higher brain functions such as memory and cognition (Incalzi *et al.* 1993; Kogure & Kato, 1993; Koistinaho *et al.* 1996). Patients suffering, for example, from chronic obstructive pulmonary disease show clear signs of dementia (Incalzi *et al.* 1993). However, even more striking is the increased incidence of dementias, particularly Alzheimer's disease, in patients who have previously suffered prolonged, severe hypoxic or ischaemic episodes arising as a consequence of cardiovascular dysfunction such as stroke or arrhythmia (Tatemichi *et al.* 1994; Kokmen *et al.* 1996; Moroney *et al.* 1996). Ischaemia causes numerous changes, including lack of metabolic substrates, accumulation of metabolic products, acidosis and reduction of ATP and O_2 levels, each of which is essential to cellular homeostasis. However, the influence of each of these parameters on cellular dysfunction is poorly understood. Nevertheless, the clear link between hypoxic/ischaemic episodes and increased incidence of dementias strongly suggests that one or more of these parameters are capable of precipitating such diseases. Recent findings, detailed below, strongly suggest that hypoxia *per se* is capable of precipitating many of the features of the most common form of dementia, Alzheimer's disease.

Alzheimer's disease is a progressive, neurodegenerative disease, which usually begins after the age of 65, but can occur much earlier due to a variety of familial mutations of proteins associated with the disease. It is characterized initially by memory decline, leading to memory loss, language deterioration, impaired visuospatial skills, poor judgment and indifferent attitude, although motor function is preserved. Over several years, cognition, personality, and the ability to function are destroyed. In the United States, it is now estimated that 4 million people have Alzheimer's disease and 100 000 people die of the disease each year. Post mortem, the disease is characterized primarily by the presence of senile plaques: small, discreet regions consisting of fibrillar amyloid β peptide (AβP) deposits, around which are regions of inflammation, cell destruction and death.

AβPs are small peptides (usually 40–42 amino acids in length) formed by the proteolytic cleavage of a much larger, membrane-spanning protein known as amyloid precursor protein (APP; Fig. 3). APP is mostly cleaved by an enzyme known as α secretase to liberate the soluble fragment, sAPPα. The cleavage site is within the AβP sequence, and so α secretase activity precludes AβP formation (Fig. 3*A*). The sAPPα fragment is generally regarded as a neuroprotective peptide (Mattson, 1997). However, APP can also be processed by the amyloidogenic pathway, which involves sequential cleavage by β and γ secretases in order to liberate the neurodegenerative AβP associated with Alzheimer's disease (Fig. 3*B*).

APP is one of only a few gene products whose expression is increased following a period of cerebral ischaemia (Kogure & Kato, 1993; Koistinaho *et al.* 1996). Since the major cleavage product of APP, sAPPα, is neuroprotective (Mattson, 1997; Selkoe, 2001), increased expression of APP may be considered a defence mechanism against cerebral hypoxia or ischaemia. However, increased APP levels

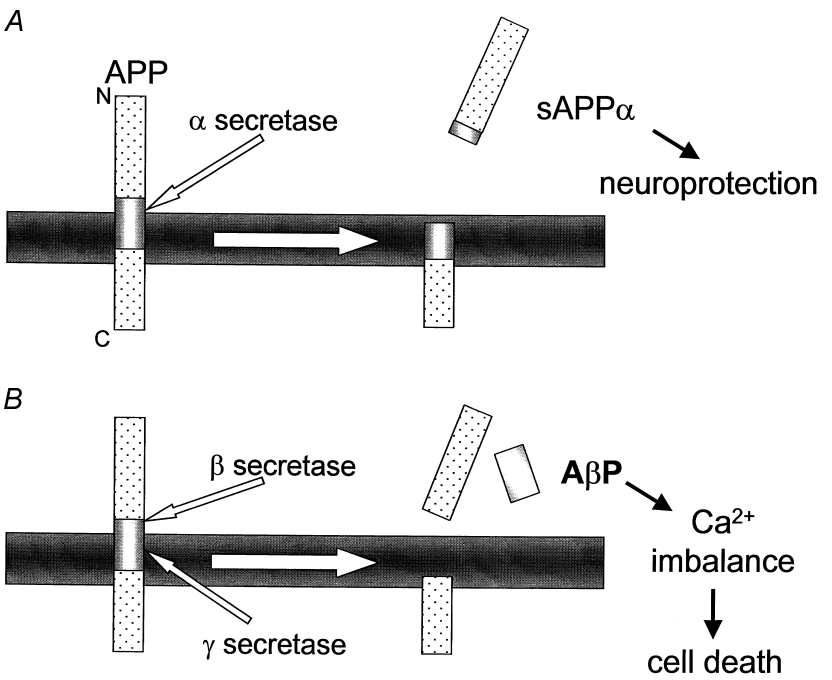

Figure 3

Schematic diagram illustrating proteolytic cleavage of amyloid precursor protein (APP). *A*, α secretase cleaves APP within the AβP sequence to liberate two peptides, including the neuroprotective sAPPα. Note that this activity prevents AβP formation. *B*, amyloidogenic processing of APP: β and γ secretases act sequentially to cleave AβP from APP, thus initiating neurodegenerative activity.

would also provide increased substrate for formation of neurotoxic AβPs through amyloidogenic processing (Fig. 3) and, indeed, AβP production is increased following periods of ischaemia in animal models (Yokota *et al.* 1996; Jendroska *et al.* 1997). These observations provide a framework for understanding the cellular and molecular basis for the increased likelihood of the onset of dementia following hypoxic/ischaemic episodes, as detailed above.

Altered Ca^{2+} homeostasis following chronic hypoxia

Despite significant recent advances in our understanding of the molecular nature of AβP production, as well as the establishment of clear lines of evidence linking hypoxic/ischaemic episodes to increased amyloidogenic processing of APP, our understanding of how this process is promoted by low O_2 levels (and so leads to an increased incidence of dementia) remains largely unexplored. Furthermore, the mechanisms underlying neuronal damage and death by AβPs remain to be clarified. Indeed, this is currently a topic of much intense research, and differing theories have been proposed. However, one important unifying feature is that the neurotoxicity of AβPs involves the disruption of Ca^{2+} homeostasis (Mattson, 1997; Selkoe, 2001).

Our own studies in this field originated with an examination of the effects of chronic hypoxia on catecholamine secretion from the phaeochromocytoma cell line, PC12 (Taylor *et al.* 1999*a*). PC12 cells are derived from rat adrenal chromaffin tissue, and had previously been established as a model system for examining cellular responses to both acute and chronic hypoxia (Zhu *et al.* 1996; Conforti & Millhorn, 1997; Taylor *et al.* 1999*b*). We found that depolarization-evoked quantal catecholamine secretion from individual PC12 cells, as monitored in real time amperometrically, was potentiated following a 24 h period of chronic hypoxia. This secretory activity was dependent on Ca^{2+} influx via voltage-gated Ca^{2+} channels, since removal of extracellular Ca^{2+} abolished secretion fully (Fig. 4). However, whilst application of Cd^{2+} (200 μM), a non-selective blocker of voltage-gated Ca^{2+} channels, also fully prevented secretion in normoxically cultured cells, this agent failed to do so completely in chronically hypoxic cells (Fig. 4), indicating that chronic hypoxia had induced the formation of a Cd^{2+}-resistant Ca^{2+} influx pathway coupled to exocytosis in these cells (Taylor *et al.* 1999*a*). It is important to note that co-incubation of these cells during hypoxia with Congo Red, an agent that prevents aggregation of AβPs, suppressed this Cd^{2+}-resistant Ca^{2+} influx pathway. Since AβPs are capable of aggregating and forming Ca^{2+}-permeable, membrane-spanning pores, these findings implied that hypoxia could induce an increased production of AβPs, which subsequently aggregated to form such channels. This conclusion was further supported by the fact that a monoclonal antibody, raised against the extracellular N′-terminus of AβP, also suppressed Cd^{2+}-resistant evoked exocytosis. Furthermore, the same antibody produced a marked enhancement of fluorescence when used as the primary antibody in immunofluorescence studies. Finally, direct application of AβPs to normoxically cultured cells, reproduced all of the effects of chronic hypoxia (Taylor *et al.* 1999*a*). Thus, a prolonged period of low O_2 was capable of increasing the production of amyloid peptides. These studies have provided a cellular basis for understanding the association of increased incidence of Alzheimer's disease with previous hypoxic/ischaemic episodes, as detailed above.

Subsequent electrophysiological studies revealed that the magnitude of this Cd^{2+}-resistant Ca^{2+} influx pathway was extremely small (< *ca* 10 % of the total inward Ca^{2+} current in these cells (Green & Peers, 2001). However, currents recorded in chronically hypoxic cells (as well as those treated with AβPs) were markedly greater in amplitude – an effect that was attributed to the selective up-regulation of L-type Ca^{2+} channels, which are normally expressed in these cells (Green & Peers, 2001). Thus, chronic hypoxia exerted two distinct effects on these cells, each of which probably contributes to the disturbance of Ca^{2+} homeostasis and both of which involved increased production of AβPs. It is interesting that the up-regulation of L-type Ca^{2+} channels (but not the formation of Cd^{2+}-resistant amyloid channels) could be prevented by treating cells with nuclear factor κB (NF-κB), indicating that the transcription of this channel was selectively increased by hypoxia (Green & Peers, 2002). Two further observations are worthy of note.

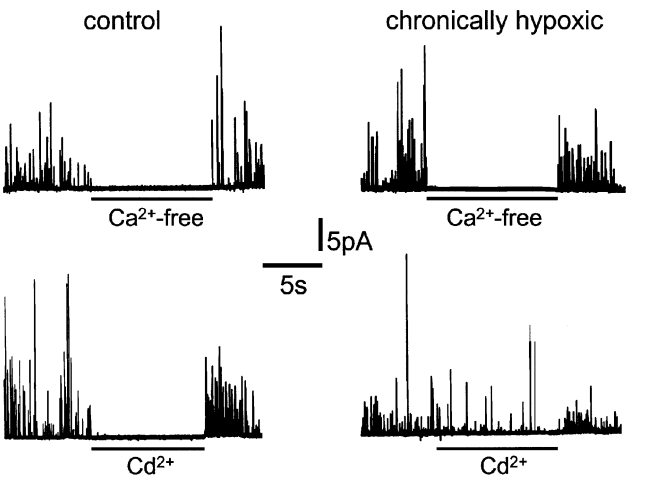

Figure 4

Amperometric detection of catecholamine secretion evoked in control (left-hand traces) and chronically hypoxic (right-hand traces) PC12 cells in response to 50 mM K^+ (applied 10 s before beginning of traces, and maintained throughout). For the periods indicated by horizontal bars, cells were exposed to either Ca^{2+}-free perfusate (replaced with 1 mM EGTA), or to 200 μM Cd^{2+} in the presence of 2.5 mM Ca^{2+} (in the continued presence of 50 mM K^+ or acute hypoxia) as indicated. Scale bars apply to all traces. Note that in chronically hypoxic cells, 200 μM Cd^{2+} failed to inhibit secretion completely. Modified from Taylor *et al.* (1999*a*).

Firstly, modulation of these two Ca^{2+} influx pathways by hypoxia appeared to *require* the formation of AβPs, since both effects were abolished when cells were cultured hypoxically, but in the additional presence of inhibitors of γ secretase (Green *et al.* 2002), one of the enzymes required for AβP formation (Fig. 3*B*). Secondly, both effects could also be prevented by antioxidants, indicating that increased production of reactive oxygen species (ROS) mediated the effects of hypoxia (Green *et al.* 2002; Green & Peers, 2002). This is perhaps not surprising to those whose interests lie in Alzheimer's disease, since AβPs have long been recognized as a potential source of ROS, arising from the interaction of methionine 35 in the amyloid sequence with metal ions such as Cu^{2+} or Al^{3+} (Behl *et al.* 1994). However, the involvement of the increased production of ROS during hypoxia is currently a subject of great interest. Schumacker and colleagues have in recent years pioneered the idea that increased ROS levels mediate O_2 sensing in a variety of tissues, and most recently have suggested that increased ROS production accounts for hypoxic pulmonary vasoconstriction (Chandel & Schumacker, 2000; Waypa *et al.* 2001). Their studies consistently point to mitochondria as being the intracellular source of ROS during hypoxia. Our findings provide an additional source of ROS, AβPs. Thus it is conceivable that in any cell type capable of generating AβPs, consequent rises of ROS levels generated by AβPs under hypoxic conditions may initiate potentially damaging processes.

Whilst these studies (summarized in Fig. 5) concerning chronic hypoxia, production of AβPs, increased ROS levels and Ca^{2+} dyshomeostasis are in their infancy, the implications are potentially extremely important. Such effects may underlie the increased incidence of Alzheimer's disease amongst patient groups who have suffered prolonged hypoxic episodes. Such observations require future study, particularly to examine whether similar effects of chronic hypoxia occur in the central nervous system *in vivo*.

Conclusions

Reduced O_2 levels regulate ion channels in two distinctly different ways. Acute hypoxia regulates the activity of ion channels already present in the plasma membrane of the cell, and in so doing alters cellular excitability. This in turn ultimately alters $[Ca^{2+}]_i$, which regulates processes such as exocytosis or contraction. In this way cellular and tissue responses are initiated, which are designed ultimately to restore O_2 to physiologically appropriate levels. Whilst exciting advances in our understanding of these processes have been made in recent years, the molecular identity of the O_2 sensor(s) in most tissues remains to be identified. Chronic hypoxia, by contrast, exerts marked effects on the functional expression of ion channels. Such responses may form part of the adaptive (acclimatizing) response to conditions of high altitude, but may also initiate pathophysiological remodelling of ion channel functional expression that may contribute to the onset of dementias including Alzheimer's disease.

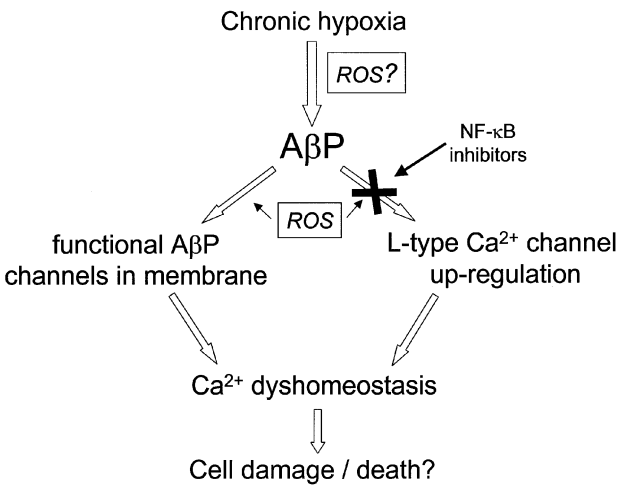

Figure 5

Schematic diagram illustrating the effects of chronic hypoxia via the increased production of amyloid β peptides (AβP) which aggregate to form Ca^{2+}-permeable plasmalemmal channels and also cause increased production of L-type Ca^{2+} channels (the latter involving regulation of transcription, since it is blocked by NF-κB). Both factors are likely to contribute to Ca^{2+} dyshomeostasis, a key step in neurodegeneration associated with Alzheimer's disease.

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