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A central role for ROS in the functional remodelling of L-type Ca^{2+} channels by hypoxia

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Periods of prolonged hypoxia are associated clinically with an increased incidence of dementia, the most common form of which is Alzheimer's disease. Here, we review recent studies aimed at providing a cellular basis for this association. Hypoxia promoted an enhanced secretory response of excitable cells via formation of a novel Ca^{2+} influx pathway associated with the formation of amyloid peptides of Alzheimer's disease. More strikingly, hypoxia potentiated Ca^{2+} influx specifically through L-type Ca^{2+} channels in three distinct cellular systems. This effect was post-transcriptional, and evidence suggests it occurred via increased formation of amyloid peptides which alter Ca^{2+} channel trafficking via a mechanism involving increased production of reactive oxygen species by mitochondria. This action of hypoxia is likely to contribute to dysregulation of Ca^{2+} homeostasis, which has been proposed as a mechanism of cell death in Alzheimer's disease. We suggest, therefore, that our data provide a cellular basis to account for the known increased incidence of Alzheimer's disease in patients who have suffered prolonged hypoxic episodes.

Keywords: hypoxia; calcium channel; Alzheimer's disease; reactive oxygen species

1. HYPOXIA AND ALZHEIMER'S DISEASE: THE CLINICAL EVIDENCE

Adaptation to conditions of chronic hypoxia is usually only seen in healthy individuals when they spend prolonged periods of time at high altitude. However, arterial O_2 levels can be markedly reduced at sea level in patients suffering from a wide variety of cardio-respiratory diseases. Examples include congestive heart failure, chronic obstructive pulmonary disease and emphysema. Prolonged periods of hypoxia can lead to damage of higher brain functions such as memory and cognition and indeed, a wealth of clinical reports indicate clearly that in such individuals the likelihood of subsequently developing dementias—particularly Alzheimer's disease—is greatly increased (Incalzi *et al.* 1993; Tatemichi *et al.* 1994; Kokmen *et al.* 1996; Moroney *et al.* 1996). Our limited understanding of this important clinical link is that it appears to occur because hypoxia can promote selectively the expression of genes which encode key proteins associated with Alzheimer's disease (Kogure & Kato 1993; Koistinaho *et al.* 1996). However, the mechanisms underlying such effects, and their physiological consequences, have not, to date, been studied in depth and so our current awareness is poor. Nevertheless, the clear link between hypoxic/ischaemic episodes and increased incidence of Alzheimer's disease strongly suggests that one or more of these parameters are capable of precipitating this increasingly widespread disease.

2. HYPOXIA AND ALZHEIMER'S DISEASE: EVIDENCE FOR CELLULAR MECHANISMS

Our previous studies, along with those of others, have shown that prolonged hypoxia causes dramatic changes in the functional expression of key proteins (particularly ion channels) in neuronally derived cells and cell lines (Wyatt *et al.* 1995; Taylor *et al.* 1999; Green & Peers 2001; Peers & Kemp 2001; Colebrooke *et al.* 2002) and also, more recently, in primary cultures of central neurons (Plant *et al.* 2002). Most importantly, we have shown that many of these effects of hypoxia involve formation of amyloid β peptides (AβPs) associated with Alzheimer's disease and can be mimicked by exposing cells to AβPs under normoxic conditions. Indeed, these dramatic effects of hypoxia appear to *require* formation of AβPs (Taylor *et al.* 1999; Green & Peers 2002; Green *et al.* 2002). Thus, of all the parameters required for normal cell function that can be disturbed during hypoxic/ischaemic episodes (e.g. lack of substrates, acidosis, accumulation of metabolic waste products) reduction in available O_2 appears to be a crucial factor in the initiation of Alzheimer's disease.

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One contribution of 18 to a Theme Issue 'Reactive oxygen species in health and disease'.

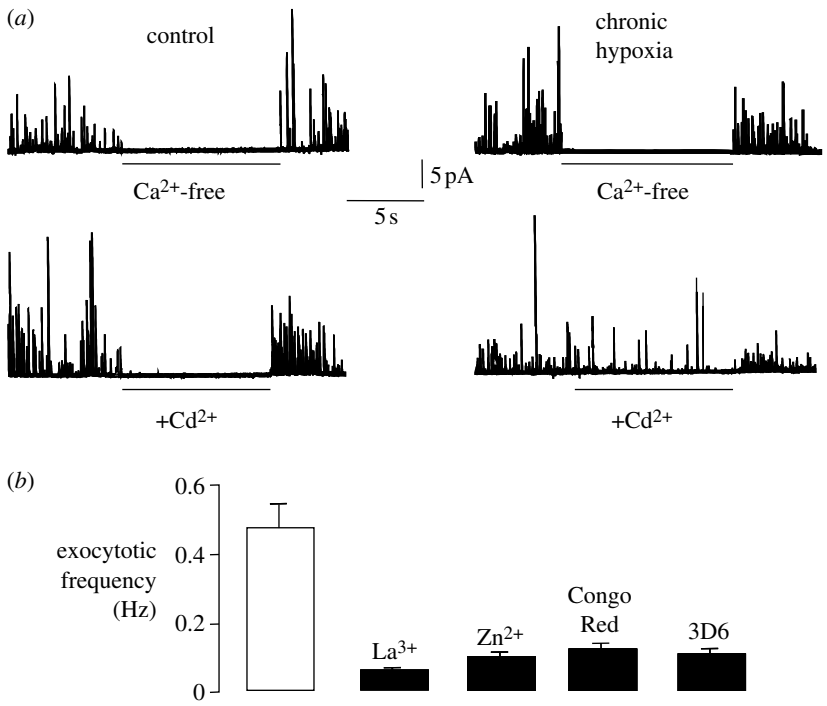

Figure 1. (*a*) Amperometric detection of secretion evoked in control (left traces) and chronically hypoxic (right) PC12 cells in response to 50 mM K^+ (stimulus application began 10 s before beginning of traces). For the periods indicated by horizontal bars, cells were exposed to either Ca^{2+}-free perfusate (upper traces), or to 200 μM Cd^{2+} (lower traces) in the presence of 2.5 mM Ca^{2+} (also in the continued presence of 50 mM K^+) as indicated. Note that in chronically hypoxic cells, 200 μM Cd^{2+} failed to inhibit secretion completely. (*b*) Bar graph showing mean (+s.e.m. bars) exocytotic frequency recorded in cells previously exposed to 10% O_2 for 21–26 h, in response 50 mM K^+ in the presence of 200 μM Cd^{2+} alone (open bar), or following further blockade with Zn^{2+} (10 mM), La^{3+} (1 mM) Congo Red (10 mM) or 3D6 antibody (5 μg ml^{-1}) as indicated. All blockers produced significant inhibition ($p<0.04$–0.001) of Cd^{2+}-resistant release ($n=8$–12 recordings in each case).

3. HYPOXIA AND EXOCYTOSIS

A wealth of evidence suggests that neuronal cell death in Alzheimer's disease arises from disruption of Ca^{2+} homeostasis. We first provided potential support for this in the actions of chronic hypoxia when examining exocytosis from individual PC12 cells, which release catecholamines in response to depolarizing stimuli that is dependent on voltage-gated Ca^{2+} entry. Exocytosis was monitored in real time, using single cell amperometry (Wightman *et al.* 1991; Finnegan *et al.* 1996). As shown in figure 1*a*, secretion (stimulated by exposure of cells to 50 mM K^+) was entirely dependent on Ca^{2+} influx, since removal of Ca^{2+} from the extracellular solution fully and reversibly prevented exocytosis. This was the case in cells cultured normoxically and also in cells cultured under hypoxic conditions (figure 1*a*, upper traces). However, when Ca^{2+} entry via voltage-gated Ca^{2+} channels was prevented by application of 200 μM Cd^{2+}, a non-selective Ca^{2+} channel blocker, a significant amount of exocytosis was evident in hypoxic (but not normoxic) cells. Thus, hypoxia induced a Cd^{2+}-resistant Ca^{2+} influx pathway coupled to catecholamine secretion.

To examine the Cd^{2+}-resistant Ca^{2+} influx pathway pharmacologically, we examined secretion in the presence of Cd^{2+} but in the additional presence of other compounds. La^{3+} and Zn^{2+}, known blockers of various Ca^{2+} influx pathways in other cells, suppressed secretion above that seen in the presence of Cd^{2+} alone (figure 1*b*). Secretion was also further suppressed by Congo Red; both Congo Red and Zn^{2+} have been shown previously to inhibit AβP-mediated Ca^{2+} fluxes (Lorenzo & Yankner 1994; Rhee *et al.* 1998), suggesting the surprising possibility that a 12–24 h period of hypoxia induced formation of Ca^{2+}-permeable amyloid peptide channels which coupled closely to exocytosis. In further support of this, we found that exposure of cells to a monoclonal antibody (3D6) raised against the extracellularly located N′ terminus of amyloid peptides (anti-$AβP_{1–5}$; Johnson-Wood *et al.* 1997) for 1 h at 37 °C also significantly suppressed the Cd^{2+}-resistant component of the secretory response (figure 1*b*). These findings strongly suggested that hypoxia caused formation of Ca^{2+} permeable channels which were tightly coupled to exocytosis and are in some way associated with amyloid peptide formation.

4. HYPOXIA AND NATIVE Ca^{2+} CHANNELS

Clearly, the implied amyloid-related Ca^{2+} entry pathway required further study. We therefore used whole-cell patch clamp recordings to monitor Ca^{2+} influx directly, and we identified a marked increase in whole cell Ca^{2+} current density in cells cultured under hypoxic conditions (2.5% O_2, 24 h), as compared with normoxic, control cells (figure 2*a*,*b*). Interestingly, this effect of hypoxia could be mimicked by exposure of cells to amyloid peptides, either $Aβ_{(1–40)}$ (figure 2*c*) or $Aβ_{(25–35)}$ (figure 2*d*). This was in accordance with the studies on exocytosis (figure 1). However, when cells were exposed to Cd^{2+} currents were virtually completely blocked (figure 2), suggesting that any Cd^{2+}-resistant Ca^{2+} influx pathway was extremely small.

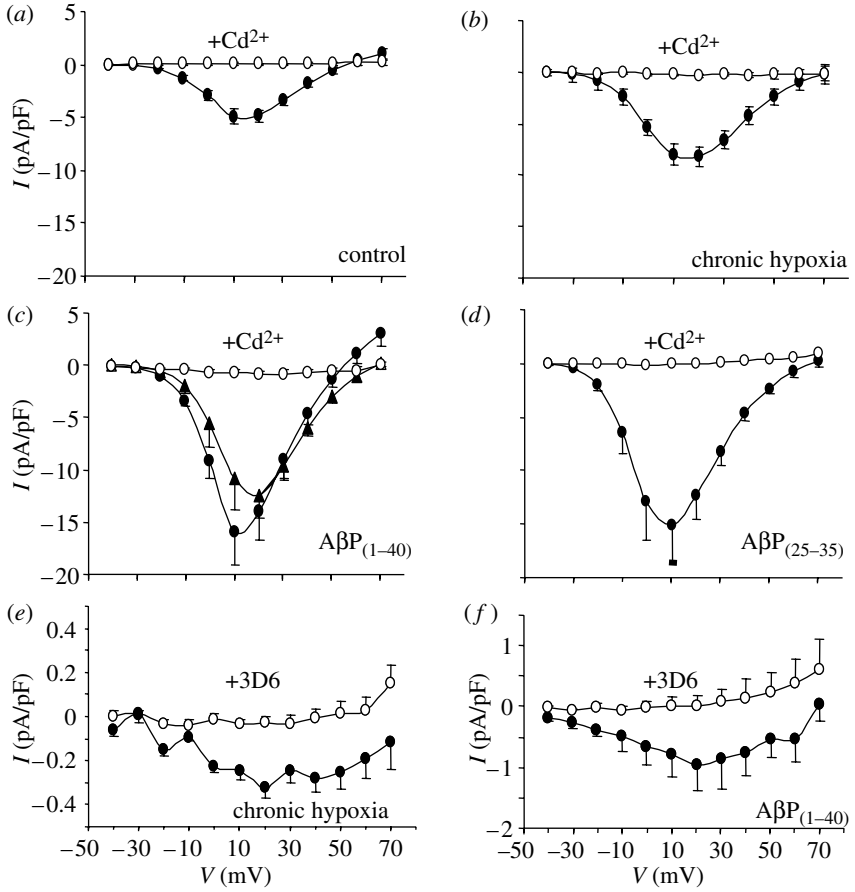

Figure 2. Augmentation of Ca^{2+} channel currents in PC12 cells by hypoxia and AβPs. Mean (+s.e.m. bars) Ca^{2+} channel current–voltage (*I–V*) relationships obtained from PC12 cells in the absence (solid circles) and presence (open circles) of 200 μM Cd^{2+}. (*a*) *I–V* relationships obtained from 20 control (normoxically cultured) cells. (*b*) *I–V* relationships obtained from 28 cells maintained in 10% O_2 for 24 h prior to recordings. (*c*) *I–V* relationships from nine cells exposed to 100 nM $AβP_{1–40}$ for 24 h prior to recording (also shown are mean data from 14 cells exposed to 20 nM $AβP_{1–40}$ for 24 h, in the absence of Cd^{2+}; solid triangles). (*d*) *I–V* relationships from nine cells exposed to $AβP_{25–35}$ for 24 h. (*e*) Mean *I–V* relationships taken from hypoxically cultured cells, recorded in the presence of 200 μM Cd^{2+} (solid circles). Open circles show data from identically treated cells, except that they were exposed to the 3D6 antibody (5 μg ml^{-1}; 1 h). (*f*) As in (*e*), except that cells were exposed to amyloid petides (as in (*c*)) rather than hypoxia ($n=8–15$ cells in each case).

Closer inspection revealed this to be the case: figure 2*e*,*f* illustrates mean Ca^{2+} currents recorded in the presence of Cd^{2+} in hypoxic (figure 2*e*) and $Aβ_{(1–40)}$ (figure 2*f*) treated cells. Note that this current could be fully blocked by the 3D6 monoclonal antibody. Note also that these tiny currents could not account for the marked increase in total whole-cell Ca^{2+} current, indicating that an additional effect of hypoxia (and amyloid) must have occurred.

PC12 cells, like many excitable neuronal or neuroendocrine cells, possess multiple types of voltage-gated Ca^{2+} channels. Of these, we found that hypoxia appeared to selectively increase the L-type Ca^{2+} channels in these cells, as reflected in the increased sensitivity to 2 μM nifedipine (figure 3*a*,*b*). This was also the case in cells treated with amyloid peptides (figure 3*c*,*d*). It was noteworthy that the nifedipine-resistant current densities in each cell group were similar. Thus, hypoxia (and amyloid peptides) selectively augmented L-type Ca^{2+} currents.

While such effects were interesting, they were somewhat removed from the question of development of Alzheimer's disease, since they were performed in a clonal cell line. Thus, to address a more pertinent cell type, we examined this phenomenon in cerebellar granule neurons (CGNs). Figure 4*a* indicates that, as in PC12 cells, Ca^{2+} currents recorded in CGNs were augmented by a period of hypoxia, and this effect was not seen when the L-type component of the Ca^{2+} current was blocked by nifedipine (figure 4*b*). At the same time, immunoreactivity for amyloid β peptide was markedly enhanced in these cells (figure 4*c*), an effect which was fully blocked by inhibition of either β- or γ-secretase, the two enzymes required for cleavage of Aβ from its precursor protein, APP (figure 4*c*). This was a primary indication that the effects of hypoxia required Aβ formation. Amyloid immunoreactivity was examined using the monoclonal antibody, 3D6 (Johnson-Wood *et al.* 1997).

5. HYPOXIA AND RECOMBINANT Ca^{2+} CHANNELS

This phenomenon of hypoxia-induced increases in functional L-type Ca^{2+} channel expression was also faithfully reproduced in a recombinant expression system. Thus, using a HEK-293 cell line stably expressing the major subunit of the human L-type Ca^{2+} channel ($α_{1C}$) in the absence of auxiliary subunits, whole-cell patch clamp recordings showed

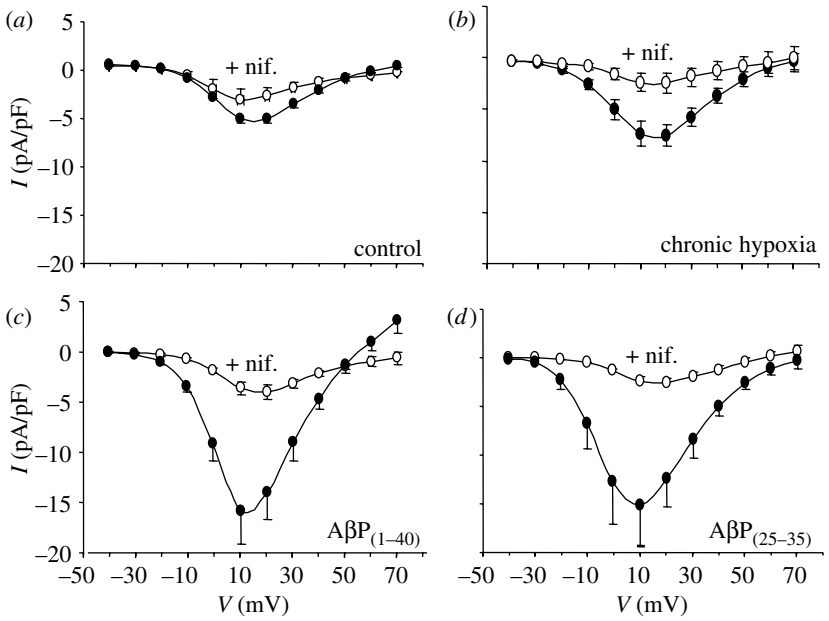

Figure 3. Nifedipine sensitivity of Ca^{2+} channel currents in PC12 cells. Mean (+s.e.m. bars) Ca^{2+} channel current–voltage (*I–V*) relationships obtained from PC12 cells before (filled circles) and during (open circles) bath application of 2 μM nifedipine. Cells were either cultured normoxically (*a*, $n=10$ cells), exposed to chronic hypoxia for 24 h (*b*, $n=8$), exposed to 100 nM $A\beta P_{1-40}$ for 24 h (*c*, $n=9$), or to 100 nM $A\beta P_{25-35}$ for 24 h (*d*, $n=9$).

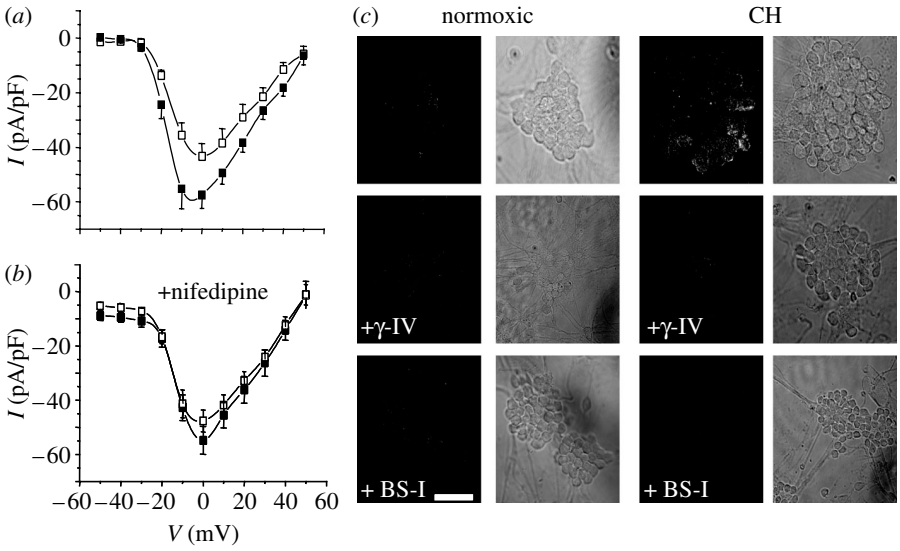

Figure 4. Hypoxia augments voltage-gated Ca^{2+} channels in cerebellar granule neurons. (*a*) Mean (+s.e.m. bars) current density versus voltage relationships obtained from neurons cultured under normoxic (open squares; $n=13$ cells) or chronically hypoxic (solid squares; $n=17$ cells) conditions. (*b*) Mean (+s.e.m. bars) current density versus voltage relationships obtained from neurons cultured under normoxic (open squares; $n=12$ cells) and chronically hypoxic (solid squares; $n=14$ cells) conditions, and recorded in the presence throughout of 2 μM nimodipine. (*c*) Immunofluorescent images of clusters of cerebellar granule cell bodies (together with bright field images, to the right of each) cultured either normoxically (left) or under chronically hypoxic conditions (right) in the absence of secretase inhibitors (top row) or in the presence of the γ-secretase inhibitor γ-IV (3 μM; middle row) or the β-secretase inhibitor, BS-I (30 nM; bottom row). Scale bar shown in the bottom of the figure indicates 10 μm and applies to all panels.

current augmentation by either hypoxia (figure 5*a*) or by AβPs (figure 5*b*). Furthermore, the effect of hypoxia could be almost fully inhibited by co-incubation with inhibitors of either β- or γ-secretases (figure 5*c*), again indicating that the effects of hypoxia required amyloid formation. This finding suggested that hypoxia did not augment current amplitudes via transcriptional regulation of the channel protein, since such transcription was under the control of the promoter regions of the plasmid introduced artificially into the HEK-293 cells. We reasoned, therefore, that increased functional expression might be attributed to a post-transcriptional event such as altered trafficking. Initial studies using double immunohistochemistry suggested that amyloid peptides and the α_{1C} subunit co-localized, particularly under hypoxic conditions (Scragg *et al.* 2005). To probe further the possible association of these two proteins, we performed immunoprecipitation and western blot experiments. Figure 6*a* shows blots taken from homogenates precipitated using the 3D6

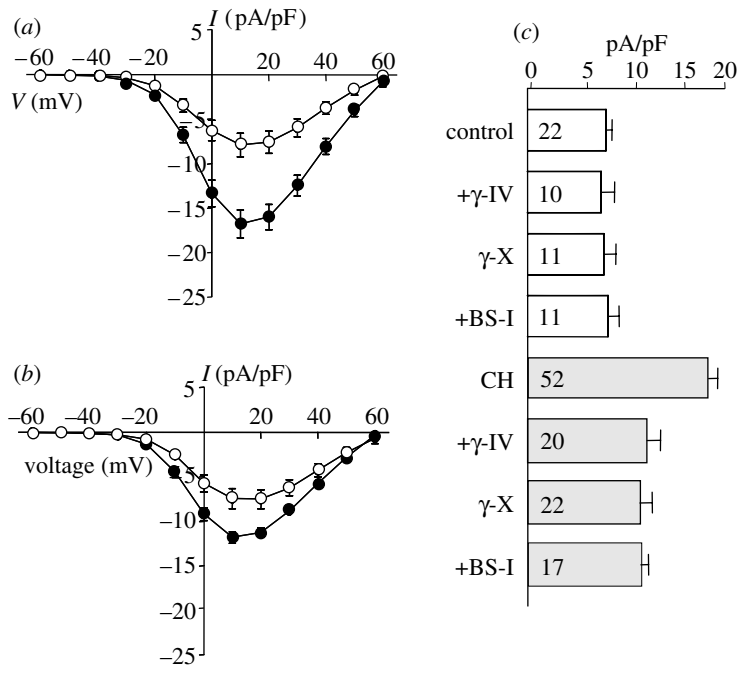

Figure 5. Hypoxia enhances Ca^{2+} channel currents in HEK-293 cells stably expressing the human cardiac L-type Ca^{2+} channel α_{1C} subunit. (*a*) Mean (+s.e.m. bars) current density versus voltage relationships obtained from control cells (open symbols, $n=12$) and from cells cultured in a hypoxic atmosphere of 2.5% O_2 for 24 h prior to recording (solid symbols, $n=15$). (*b*) Mean current density (+s.e.m. bars) versus voltage relationships obtained from control cells (open symbols, $n=9$) and from cells pretreated for 24 h with 20 nM $A\beta P_{1-40}$ (solid symbols circles, $n=18$). (*c*) Bar graph showing mean (+s.e.m.) current density (determined at a test potential of +20 mV) in control cells (open bars) and in hypoxically cultured (CH) cells (shaded bars) exposed to various secretase inhibitors (the β-secretase inhibitor, BS-I (30 nM), the γ-secretase inhibitor, γ-IV (2.5 μM) and the γ-secretase inhibitor, γ-X (100 nM), as indicated). Values in bars indicate *n* numbers.

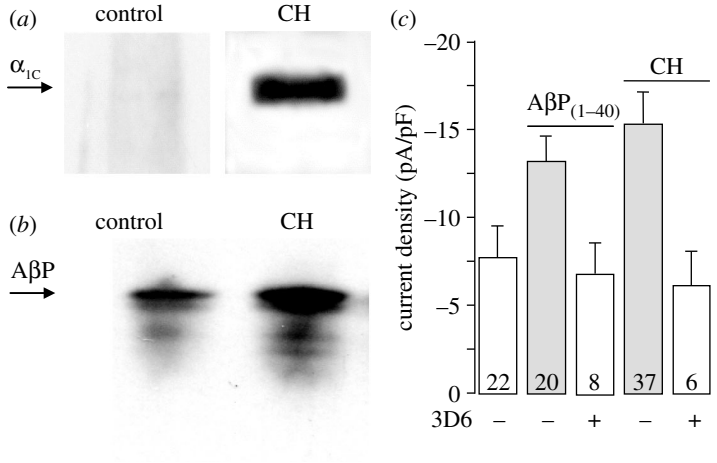

Figure 6. Evidence for co-localization of amyloid peptide and Ca^{2+} channels. (*a*) Western blots probed with the anti-α_{1C} antibody, following immunoprecipitation of cell homogentates using the 3D6 monoclonal anti-amyloid antibody. Note marked immunodetection in hypoxic but not normoxic cells. Representative of six experiments. (*b*) Detection of AβPs in samples of control and hypoxic cell homogenates, immunoprecipitated using the anti-α_{1C} antibody as indicated. Blots were probed using the 3D6 antibody. Representative of six experiments. (*c*) Bar graph plotting mean (+s.e.m.) current density observed at a test potential of +20 mV in control cells, cells exposed to 20 nM $A\beta P_{1-40}$ for 24 h, and cells exposed to chronic hypoxia (as indicated) either with (open bars) or without (shaded bars) subsequent exposure to the anti-amyloid peptide monoclonal antibody 3D6 (5 μg ml^{-1}). Values in bars indicate *n* numbers.

(anti-amyloid) antibody then probed with the anti-α_{1C} antibody. In control cells (left), the signal was extremely weak. However, a clear band of appropriate molecular weight was detected in hypoxic cells (right). These results (representative of six paired experiments) were supported in experiments where homogenates were precipitated with the anti-α_{1C} antibody then probed using the 3D6 antibody (figure 6*b*). In this case, amyloid peptides were detected in both control (figure 6*b*, left) and hypoxic (right) cell samples, proof of physical interaction under both conditions, but band intensity was consistently ($n=6$ experiments) greater in hypoxic samples.

Figure 6*c* plots mean current densities (taken at the membrane potential at which currents were maximal in amplitude) in control cells, and those cultured under

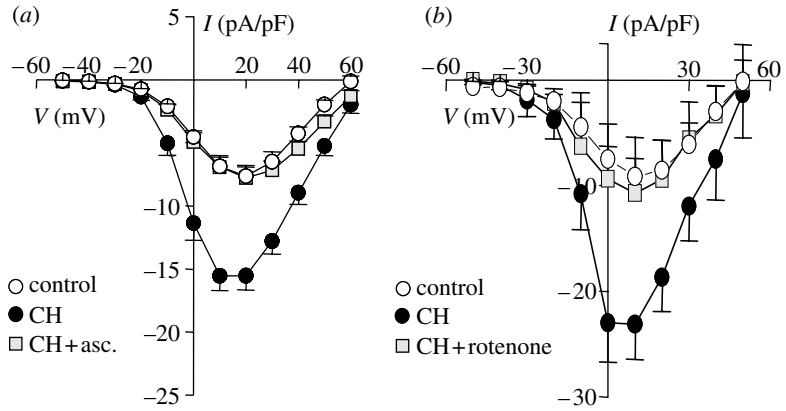

Figure 7. Involvement of mitochondrial ROS in hypoxic augmentation of Ca^{2+} channels. (*a*) Mean (+s.e.m.) current density–voltage relationships obtained in HEK-293 cells stably expressing L-type Ca^{2+} channel α_{1C} subunits. Cells were cultured under normoxic conditions (open circles), hypoxic conditions (solid circles) or hypoxic conditions in the additional presence of 200 μM ascorbic acid (asc., squares). Data were obtained between 7 and 12 cells for each experimental condition. (*b*) Mean (+s.e.m.) current density–voltage relationships obtained in HEK-293 cells stably expressing L-type Ca^{2+} channel α_{1C} subunits. Cells were cultured under normoxic conditions (open circles), hypoxic conditions (solid circles) or hypoxic conditions in the additional presence of 1 μM rotenone (squares). Data were obtained between 8 and 30 cells for each experimental condition.

hypoxic conditions or in the presence of amyloid peptides. Also shown are data obtained from hypoxic or amyloid-treated cells following a 1 h period in which cells were also exposed to 5 $\mu g\,ml^{-1}$ of the 3D6 monoclonal antibody. The antibody fully reversed current augmentation under either experimental condition. This effect was comparable to the effects of autoantibodies present in the plasma of patients with Lambert–Eaton myasthenic syndrome (Vincent *et al.* 1989), who present with muscular weakness due to suppression of acetylcholine (ACh) release from motor nerve terminals. ACh release is suppressed because autoantibodies bind to, and cross-link, presynaptic Ca^{2+} channels (Vincent *et al.* 1989; Peers *et al.* 1993). This cross-linking in turn triggers endocytotic down-regulation of channels. With this in mind, an attractive (though still speculative) explanation for the present studies is that 3D6 antibodies also trigger down-regulation of α_{1C} subunits via cross-linking of adjacent amyloid peptides which our co-immunoprecipitation experiments (figure 6*a*,*b*) suggest are tightly coupled.

6. THE INVOLVEMENT OF REACTIVE OXYGEN SPECIES IN HYPOXIC CURRENT AUGMENTATION

A growing body of evidence suggests that hypoxia can, seemingly paradoxically, cause an increase in the generation of reactive oxygen species (ROS; e.g. Duranteau *et al.* 1998; Chandel & Schumacker 2000; Waypa & Schumacker 2005). The ROS appear to be derived specifically from mitochondria. To examine whether ROS were involved in hypoxia-induced increases of Ca^{2+} channel currents, we examined the effects of antioxidants. Results are exemplified by the action of ascorbic acid (figure 7*a*), which fully prevented the augmentation of currents by hypoxia, while leaving control (normoxic) currents unaffected (not shown). Furthermore, this inhibitory effect of ascorbic acid was mimicked by co-culturing cells under hypoxic conditions in the additional presence of rotenone, an inhibitor of complex I of the mitochondrial electron transport chain. Thus, again, hypoxic augmentation of currents was fully blocked (figure 7*b*). These findings are in accordance with the suggestion of Schumacker and colleagues (e.g. Chandel & Schumacker 2000; Waypa & Schumacker 2005) that hypoxia increases ROS production from mitochondria.

7. CONCLUSIONS

The data presented herein provide a plausible cellular basis for the known association of prolonged hypoxia and dementia, as documented in several clinical studies (Incalzi *et al.* 1993; Tatemichi *et al.* 1994; Kokmen *et al.* 1996; Moroney *et al.* 1996). In PC12 cells, we noted two distinct effects of hypoxia—the induction of a Cd^{2+}-resistant Ca^{2+} influx pathway which was tightly coupled to exocytosis, and the selective upregulation of L-type Ca^{2+} channels. Both effects involve amyloid β peptide formation. Indeed, where tested, the effects of hypoxia *require* amyloid formation, since inhibitors of either β- or γ-secretases (the enzymes required for cleavage of amyloid peptide from its precursor; Mattson 1997; Vassar & Citron 2000; LaFerla 2002) prevented effects of hypoxia.

The induction of an amyloid-associated influx pathway coupled to exocytosis has not been explored further in detail, but is reminiscent of amyloid-mediated ion channel activity previously reported (Arispe *et al.* 1993, 1996; Pollard *et al.* 1993). These previous studies have suggested that small aggregates of amyloid peptides can form membrane-spanning, Ca^{2+} permeable ion channels, thereby contributing to the dysregulation of Ca^{2+} homeostasis believed to be involved in neuronal cell death in Alzheimer's disease (LaFerla 2002). Further studies are require to explore this possibility but it is important to note that hypoxic induction of this influx pathway is distinct from the effect of hypoxia to upregulate native L-type Ca^{2+} channels. The distinction is emphasized by our findings that the influx pathway coupled to secretion was unaffected by inhibitors of the transcriptional regulator NFκ-B, whereas the enhancement of L-type Ca^{2+} channels was not (Green & Peers 2002).

The most striking effect of hypoxia was the enhancement of L-type Ca^{2+} channels, a phenomenon which was observed not only in PC12 cells, but also in central neurons and a recombinant expression system. This in itself suggests the effect is not tissue specific and so is likely to be a widespread effect. Most importantly, in all cell types this effect of hypoxia required amyloid formation. It is this requirement which convinces us that Ca^{2+} channel upregulation provides a cellular basis for the clinical association of hypoxia with dementia (see above). L-type Ca^{2+} channels serve a variety of important functions (Lipscombe *et al.* 2000; Striessnig *et al.* 2004; Wang *et al.* 2004), and so their selective targeting by amyloid peptides is likely to have profound (and deleterious) effects on cell activity and survival.

The fact that we could reproduce the effects of hypoxia on L-type Ca^{2+} channels in a recombinant expression system allowed us to draw immediate conclusions. First, channel upregulation was unlikely to occur via increased channel gene transcription, since the gene was part of a foreign plasmid incorporated into these cells which contained no known hypoxia-sensitive regions. Second, channel upregulation did not require the presence of auxiliary subunits. Normally L-type (and other) Ca^{2+} channels are protein complexes, with auxiliary subunits regulating normal channel activity (Dolphin 1995; Yamaguchi *et al.* 1998). Our recombinant expression system lacked auxiliary subunits, yet amyloid mediated hypoxic enhancement of currents was striking.

Looking for post-transcriptional mechanisms, we explored the possibility that amyloid peptides might act as chaperone molecules, altering the trafficking of Ca^{2+} channels. Evidence to support this idea came from a striking increase in the co-localization of the two proteins, as determined by double-labelling immunocytochemistry (Scragg *et al.* 2005). Furthermore, the two proteins co-immunoprecipitated (figure 6), suggesting they physically interact (although how direct this is, we cannot currently determine). We propose that this association is also present at the plasma membrane, since exposure of hypoxic (or amyloid-treated) cells to the 3D6 antibody raised against amyloid peptide suppressed any enhancement of current amplitude.

Importantly, we also provided evidence that the effects of hypoxia require formation of ROS. This is a contentious issue, since some groups believe ROS to increase during hypoxia (Waypa & Schumacker 2005), while others believe ROS levels to decrease in hypoxia (Michelakis *et al.* 2004), even when studied in the same tissue. The latter opinion is the more instinctive, since ROS are derived from O_2, yet compelling evidence is accumulating for the former. Our findings are consistent with the idea that increased production of ROS is required for the observed effects of hypoxia on Ca^{2+} channels. Thus, not only do antioxidants prevent any effects of hypoxia, but exposure of cells to H_2O_2 mimics the effects of hypoxia (Green *et al.* 2002). Furthermore, hypoxic ROS generation appears to be from mitochondria (figure 7), in accordance with other studies (Waypa & Schumacker 2005).

A key fundamental question is how hypoxia triggers increased amyloidogenic processing (i.e. the formation of amyloid peptides from their precursor protein, APP). We are currently exploring the alternative processing pathways of APP, and our recent findings suggest that increased amyloid formation might arise due to increased expression of presenilin-1, the major component of γ-secretase, essential for amyloid formation (Smith *et al.* 2004). Clearly there is much further work to be done, but our data to date provide a plausible cellular basis to account for the known increased incidence of Alzheimer's disease in patients who have suffered prolonged hypoxic episodes.

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