ELSEVIER

Neurobiology of Aging 27 (2006) 439–445

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Amyloid peptides mediate hypoxic increase of L-type Ca^{2+} channels in central neurones

N.J. Webster [a], M. Ramsden [b], J.P. Boyle [a], H.A. Pearson [b], C. Peers [a,*]

[a] *Institute for Cardiovascular Research, Schools of Medicine, University of Leeds, Leeds LS2 9JT, UK*
[b] *Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK*

Received 28 October 2004; received in revised form 2 February 2005; accepted 17 February 2005

Abstract

Prolonged hypoxia, encountered in individuals suffering from various cardiorespiratory diseases, enhances the likelihood of subsequently developing Alzheimer's disease (AD). However, the underlying mechanisms are unknown, as are the mechanisms of neurodegeneration of amyloid β peptides (AβPs), although the latter involves disruption of Ca^{2+} homeostasis. Here, immunohistochemistry demonstrated that hypoxia increased production of AβPs, an effect which was prevented by inhibition of either β or γ secretase, the enzymes required for liberation of AβP from its precursor protein. Whole-cell patch clamp recordings showed that hypoxia selectively increased functional expression of L-type Ca^{2+} channels. This was prevented by inhibition of either β or γ secretase, indicating that hypoxic channel up-regulation is dependent upon AβP formation. Our results indicate for the first time that hypoxia promotes AβP formation in central neurons, and show that this leads to abnormally high selective expression of L-type Ca^{2+} channels whose blockade has previously been shown to be neuroprotective in AD models. These findings provide a cellular basis for understanding the increased incidence of AD following prolonged hypoxia.

Keywords: Hypoxia; Alzheimer's disease; Ca^{2+} channels; Secretases; Patch clamp; Immunohistochemistry; Neurodegeneration

1. Introduction

One major defining feature of Alzheimer's disease (AD) is the presence of fibrillar deposits composed primarily of amyloid β peptides (AβPs), cleavage products derived from amyloid precursor protein (APP) via the sequential action of β- and γ-secretases [1,27]. Whilst our understanding of the mechanisms underlying the neurodegeneration of AD is incomplete, there is compelling evidence that AβPs are neurotoxic elements responsible for the progressive death of neurons [18,27]. The means by which AβPs exert their toxicity remains contentious, but a large consensus of opinion indicates that disruption of Ca^{2+} homeostasis is central to the action of AβPs [17,19].

Cardiorespiratory diseases such as chronic obstructive pulmonary disease or arrhythmias can lead to a marked reduction in the O_2 supply to the brain, and these conditions are all associated with a significant increase in the likelihood of developing dementias, particularly AD [11,14,15]. Even more striking is the increased incidence of AD in patients who have previously suffered prolonged hypoxic or ischemic episodes arising from a stroke [16,20,21,31].

APP is one of only a few gene products whose expression is increased following a period of cerebral hypoxia/ischemia [14,15]. Since APP (in non-AD patients) is preferentially cleaved by α secretase to liberate the neuroprotective soluble sAPPα, this increased expression of APP might be considered a defence mechanism against ischemia. However, increased APP levels could also permit increased formation of AβPs via the actions of β and γ secretases and, indeed, AβP production is increased following both mild and severe ischemia [12,37]. Thus, evidence for a clear link exists between hypoxic/ischemic insult and elevation of damaging AβP levels. We have previously demonstrated that prolonged hypoxia enhances the exocytotic response of pheochromocytoma (PC12) cells to depolarising stimuli, by enhancing Ca^{2+} influx, an effect which appeared dependent on

* Corresponding author. Tel.: +44 113 343 4174; fax: +44 113 343 4803.
E-mail address: c.s.peers@leeds.ac.uk (C. Peers).

0197-4580/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2005.02.002

hypoxia-induced increases of AβP production [32,33]. Our results suggested that hypoxia led to the formation of Ca^{2+}-permeable membrane channels consisting of AβPs, as well as up-regulation of L-type Ca^{2+} channels [6–8]. However, these studies were restricted to a clonal cell line, and effects observed in these cells cannot be extrapolated to account for effects of hypoxia in central neurones. The present study was, therefore, conducted to examine the effects of prolonged hypoxia on voltage-gated Ca^{2+} channels in primary cultures of central neurones, and to investigate any potential role of AβP formation.

2. Methods

2.1. Culturing of central neurones

All experiments were performed using primary cultures of rat cerebellar granule neurons. Cells were obtained by enzymatic and mechanical dissociation as previously described [9,25]. This preparation was selected because, although such neurones are resistant to hypoxic insult in vivo, any neurone is likely to alter protein expression during even short-term culture. These neurones, however, have the distinct advantage of expressing the widest variety of voltage-gated channels known [22,26]. Briefly, cerebellar tissue was removed from 6 to 8 day old rat pups and triturated following a 15 min trypsin (EC 4.4.21.4, 2.5 mg ml^{-1} in phosphate buffered saline) digestion. Cells, plated at a density of 0.25×10^6 cells per well on circular 10 mm diameter poly-L-lysine coated coverslips, were grown in a humidified atmosphere containing 5% CO_2: 95% air at 37 °C. For cells exposed to hypoxia, the atmosphere was adjusted to 2.5% O_2, 5% CO_2, balance N_2 for 24 h prior to experimentation. The culture medium consisted of minimal essential medium supplemented with 10% horse serum, 2.5% chick embryo extract, 26 mM glucose, 19 mM KCl, 2 mM L-glutamine, penicillin/streptomycin (50 IU ml^{-1}/50 μg ml^{-1}) and 80 μM fluorodeoxyuridine to prevent proliferation of non-neuronal cells. Culture media were exchanged every 3 days and cells were grown in culture for up to 11 days. All recordings were made from cells between days 7 and 11.

2.2. Electrophysiological recording of Ca^{2+} channel currents

For recording Ca^{2+} channel currents patch pipettes were filled with solution containing (in mM) Cs-HEPES 100, EGTA 30, $CaCl_2$ 3, $MgCl_2$ 2.5, K_2ATP 3.25; osmolarity 320 mOsmol with sucrose; pH was adjusted to 7.2 with CsOH. Cells were bathed in a solution that comprised (in mM) tetraethylammonium acetate 70, *N*-methyl-D-glucamine 70, KOH 3, magnesium acetate 0.6, glucose 4, barium acetate 10, HEPES 10, and tetrodotoxin 0.0005; pH 7.4 with acetic acid; osmolarity 320 mOsmol with sucrose. To record Ca^{2+} channel current–voltage relationships, cells were held at a potential of −90 mV and depolarised to potentials ranging from −50 to +50 mV. The steps were repeated every 10 s. Five leak subtraction steps were made prior to depolarisation to allow off-line removal of linear leak and residual capacity artefacts. Series resistance compensation of 80% was routinely employed.

Ca^{2+} channel antagonists ω-conotoxin GVIA (ω-CgTx; Peninsula Laboratories, St. Helens, UK) and nimodipine (Tocris, Bristol, UK) were made up as stock solutions in deionised water or DMSO as appropriate and were then frozen at −20 °C before appropriate dilution in the recording medium. Experiments using nimodipine were conducted in the dark due to the light sensitivity of this compound. Ca^{2+} channel blocking compounds were added to the recording chamber prior to recording and were present throughout electrophysiological measurement of channel activity. All culture reagents were obtained from Gibco BRL (UK), except chick embryo extract which was purchased from Imperial Laboratories (UK).

2.3. Analysis of electrophysiological recordings

Current recordings were analysed using the Patch v6.0 program by Cambridge Electronic Design (Cambridge, UK) following leak subtraction using a P/5 subtraction protocol. Further analyses were performed using Microsoft Excel 2000 and Microcal Origin v6.0. All data are given as mean ± standard error of the mean. Student's *t*-test (unpaired) was used to determine the significance of differences between the means, with P values <0.05 being considered significant. All current recordings were normalized to whole-cell capacitance to give current density. Peak current was measured as the maximal inward current observed during the depolarising step. Voltage errors due to series resistance were calculated and never came to more than −2 to 3 mV. Junction potential error was directly measured as +3 mV. No adjustments for these errors were therefore made.

2.4. Immunocytochemistry

Immunofluorescent labelling with a monoclonal antibody raised against the extracellular N-terminal 5 residues of AβP (3D6 antibody [13]) was performed as previously described [32] with cells plated onto coverslips and subjected to normoxic or other conditions as described above. Cells were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS; pH 7.4) for 20 min, and then rinsed thoroughly in several changes of 0.1 M PBS. The cells were then incubated for 20 min at room temperature in PBS containing 10% normal goat serum (NGS) to block non specific sites and 0.2% Triton X-100 to permeabilse the cells. Cells were then thoroughly washed again with PBS for several changes. The coverslips were then incubated with the 3D6 antibody (diluted to 0.5 μg/ml in PBS) overnight at 4 °C. After two 10 min rinses in PBS, the cells were incubated for 2 h in a 1/200 dilution of Cy2 conjugated anti-mouse IgG

(Jackson ImmunoResearch). After two further 10 min rinses in PBS, the coverslips were mounted onto glass microscope slides with glycerol/PBS and the edges of the coverslips were sealed with clear nail polish. The cells were examined using a Zeiss Axioskop epifluorescence microscope using a No. 10 (fluorescein) filter set. Photographs were taken using a Kodak MDS120 digital camera system.

3. Results

3.1. Hypoxia increases L-type Ca^{2+} current density in cerebellar granule neurones

Fig. 1A plots mean current density versus voltage relationships obtained from cerebellar granule neurones cultured under normoxic and chronically hypoxic (CH; 2.5% O_2, 24 h) conditions. Clearly, the current densities observed in CH cells were enhanced as compared with controls, and this effect was statistically significant ($P < 0.05$) at test potentials of −10 and 0 mV. No marked change in current kinetics was observed (Fig. 1A, inset). Previous studies have indicated that hypoxia can induce a Ca^{2+} influx pathway which is insensitive to the non-selective inhibitor of voltage-gated Ca^{2+} channels, Cd^{2+} [7,32]. However, as illustrated in Fig. 1B, the residual current in the presence of Cd^{2+} (200 μM) was very small and not significantly different between normoxic and CH cells, indicating that the augmentation of total whole-cell Ca^{2+} current was attributable to up-regulation of one or more endogenous voltage-gated Ca^{2+} channel types.

Cerebellar granule neurones express multiple types of voltage-gated Ca^{2+} channel [22,26]. Since previous studies had indicated a selective enhancement of L-type (Ca_v1) Ca^{2+} channels in PC12 cells by hypoxia [6,7], we explored this possibility in cerebellar granule neurons. Fig. 2A indicates that whole-cell Ca^{2+} currents, recorded in the presence of 2 μM nimodipine to block L-type channels, were unaffected by CH. By contrast, when cells were pretreated with ω-CgTx (1 μM) to block N-type ($Ca_v2.2$) Ca^{2+} channels, the effects of hypoxia were markedly enhanced. The fact that inhibition of non-L-type channels exaggerated the effects of hypoxia, whilst inhibition of L-type channels prevented effects

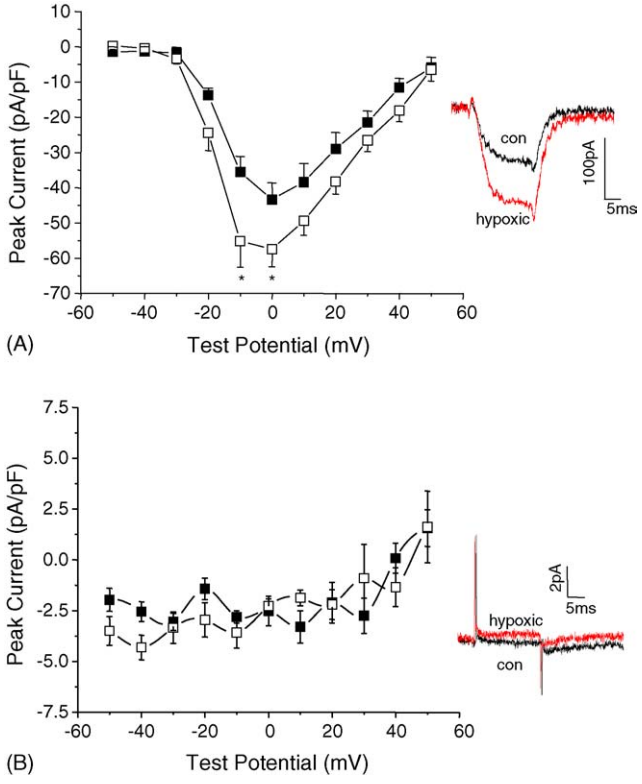

Fig. 1. Hypoxia augments voltage-gated Ca^{2+} channels in cerebellar granule neurones. (A) Mean (±S.E.M. bars) current density vs. voltage relationships obtained from neurons cultured under normoxic (■, $n = 13$ cells) and chronically hypoxic (□, $n = 17$ cells) conditions. Inset shows superimposed traces of mean currents, taken from each group of cells at a test potential of 0 mV, as indicated. (B) Mean (±S.E.M. bars) current density vs. voltage relationships obtained from neurons cultured under normoxic (■, $n = 15$ cells) and chronically hypoxic (□, $n = 15$ cells) conditions in the presence throughout of 200 μM Cd^{2+}. $^{*}P < 0.05$. Ensemble averaged currents, recorded at the 0 mV test potential for the same cells are shown on the right.

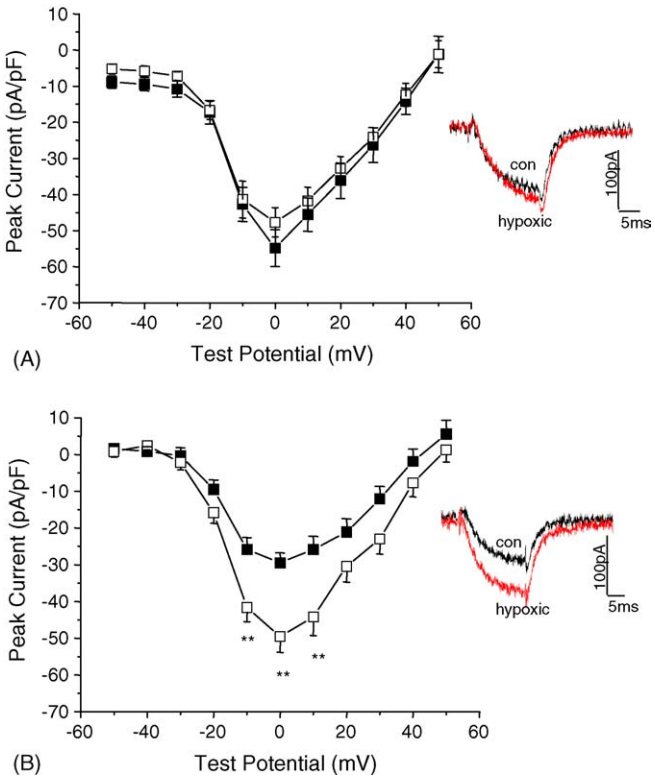

Fig. 2. Hypoxia selectively augments L-type Ca^{2+} channels. (A) Mean (±S.E.M. bars) current density vs. voltage relationships obtained from neurons cultured under normoxic (■, $n = 12$ cells) and chronically hypoxic (□, $n = 14$ cells) conditions and recorded in the presence throughout of 2 μM nimodipine. Inset shows superimposed traces of mean currents, taken from each group of cells at a test potential of 0 mV, as indicated. (B) As (A), except that cells were pre-treated with 1 μM ω-CgTx prior to recordings (normoxic; $n = 16$ cells and chronically hypoxic; $n = 17$ cells). $^{**}P < 0.01$, $^{***}P < 0.001$. Inset shows superimposed traces of mean currents, taken from each group of cells at a test potential of 0 mV.

of hypoxia, indicates strongly that the augmented current density seen in CH cells is due to a selective enhancement of L-type channels. Therefore, subsequent electrophysiological recordings (Fig. 4) were made in cells pretreated with 1 μM ω-CgTx.

3.2. Amyloid peptides mediate hypoxic augmentation of Ca^{2+} currents

As discussed earlier, previous evidence suggested that at least some of the effects of CH involve formation of AβPs. To investigate their involvement in hypoxic up-regulation of L-type Ca^{2+} channels in cerebellar granule neurons, we looked for immunohistochemical evidence of increased amyloid formation. Fig. 3 shows immunofluorescent images (together with the corresponding brightfield images) of cell body clusters cultured under normoxic and hypoxic conditions, as indicated. Clearly, as compared with normoxically cultured cells, those kept under hypoxic conditions showed a marked increase in AβP levels (top row of images). The middle row of images are taken from cells treated with the dipeptide-aldehyde, 2-naphthoyl-VF-CHO (γ-IV; 3 μM), a cell permeable inhibitor of γ-secretase activity. γ-IV reversibly inhibits production of both the 1–40 and 1–42 forms of AβP with ED_{50} values of 2.6 and 2.7 μM respectively [29]. Incubation of cerebellar granule neurones with 3 μM γ-IV during 24 h of hypoxia completely suppressed the increased immunofluorescence observed in CH cells. Similarly, cells were exposed to 30 nM H-KTEEISEVN-stat-VAEF-OH (βSI), a potent inhibitor of β-secretase activity (IC_{50} 30nM [28]). Again, the increased production of AβPs caused by hypoxia was fully prevented. These data indicate that hypoxia can stimulate production of AβPs in central neurons, and this is fully prevented by inhibition of either β or γ secretase.

We next investigated whether any association existed between hypoxia-induced increased in Ca^{2+} current density and hypoxia-induced increases in AβP formation. Fig. 4A shows that culturing cells under normoxic conditions in the presence of 3 μM γ-IV had no effect of Ca^{2+} current density. However, this inhibitor fully prevented the augmentation of current densities attributable to chronic hypoxia (Fig. 4B). Similarly, the β secretase inhibitor, βSI (30 nM), was without effect on currents in normoxically cultured cells (Fig. 4C), yet fully prevented hypoxic augmentation of currents (Fig. 4D). These electrophysiological recordings were all made in cells pretreated with ω-CgTx (1 μM) to block N-type channels and hence highlight the hypoxic up-regulation of L-type channels (see Fig. 2). Our findings therefore indicate that the functional up-regulation of L-type Ca^{2+} channels in cerebellar granule neurons requires AβP formation.

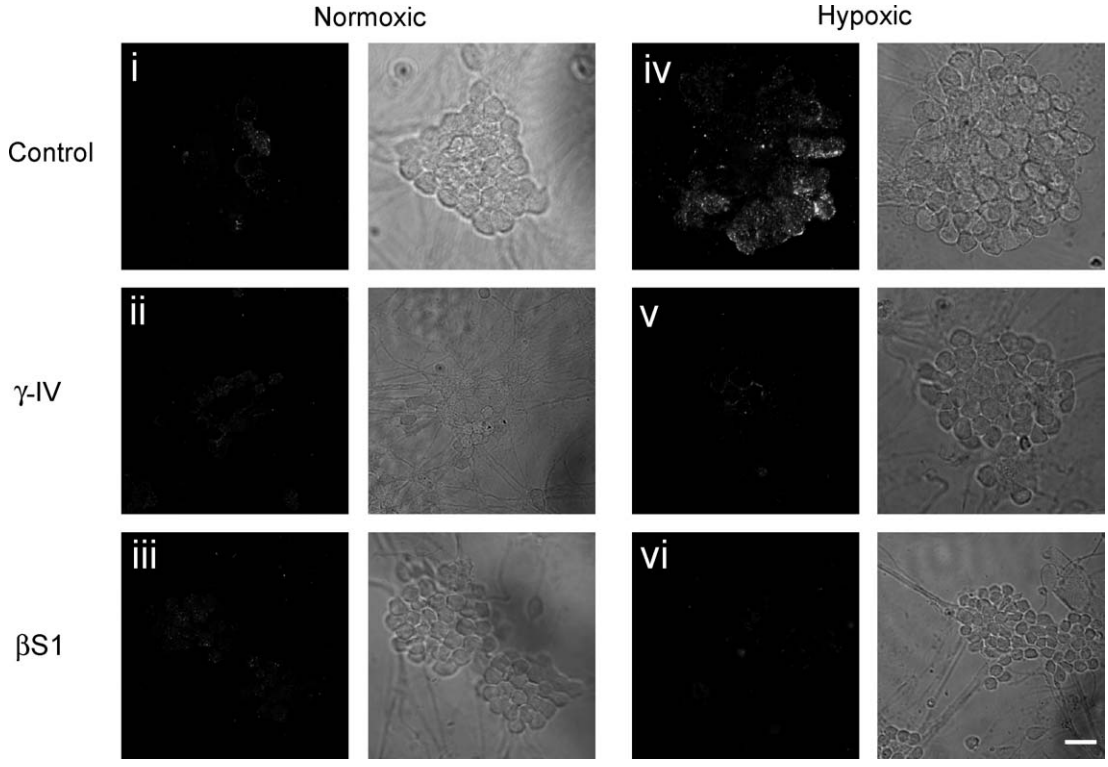

Fig. 3. Hypoxia augments AβP immunoreactivity in cerebellar granule neurons. Immunofluorescent images of clusters of cerebellar granule cell bodies (together with bright field images, to the right of each) cultured either normoxically (left) or under chronically hypoxic conditions (right) in the absence 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, βSI (30 nM; bottom row). Scale bar shown in the bottom right of the figure indicates 10 μm and applies to all panels.

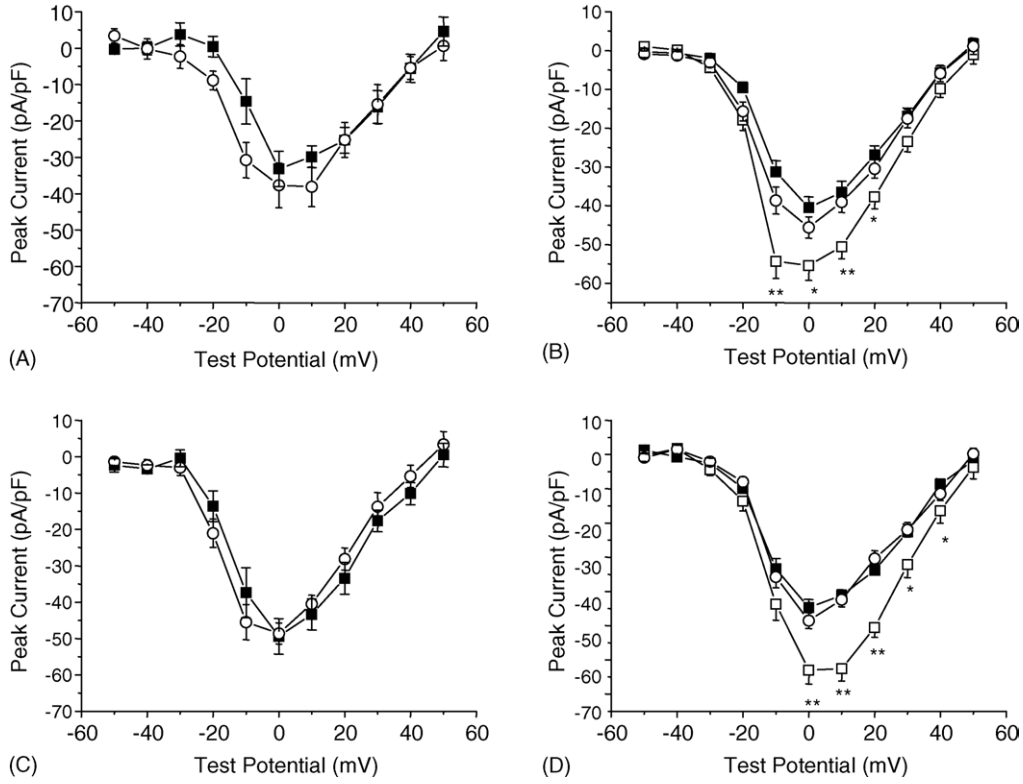

Fig. 4. Inhibition of amyloid formation prevents hypoxic augmentation of Ca^{2+} currents. (A) Mean (±S.E.M. bars) current density vs. voltage relationships obtained from neurons cultured under normoxic conditions in the absence (■, $n = 8$ cells) and presence (◯, $n = 9$ cells) of the γ secretase inhibitor γ-IV (3 μM). (B) Mean (±S.E.M. bars) current density vs. voltage relationships obtained from neurons cultured under normoxic (■, $n = 37$ cells) and hypoxic conditions in the absence (□, $n = 28$ cells) or presence (◯, $n = 33$ cells) of the γ secretase inhibitor γ-IV (3 μM). (C) as (A), except that currents were recorded in the absence or presence of the β secretase inhibitor, βSI (30 nM, $n = 14$ cells for each condition). (D) as (B) except that currents were recorded in the absence or presence of βSI (30 nM, normoxic; $n = 33$ cells, chronically hypoxic; $n = 26$ cells, and chronically hypoxic + βS1; $n = 28$ cells). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

We have previously shown that chronic hypoxia increases formation of AβPs in the clonal cell line, PC12 [32] and in primary cultures of astrocytes [30]. The present study demonstrates that hypoxia also increases formation of AβPs in primary cultures of central neurons, a finding of particular importance in attempting to understand the mechanisms underlying the increased incidence of neurodegenerative AD in individuals who have previously experienced prolonged hypoxia (see Section 1).

Our immunofluorescent studies (Fig. 2) clearly show an increased immunoreactivity for AβPs specifically in those cells cultured under hypoxic conditions. These studies were performed using the monoclonal antibody, 3D6, which was originally raised against the N′ terminal 5 residues of AβP [13]. This antibody is, therefore, capable of also recognizing the C99 peptide product yielded by the action of β secretase on APP. However, our results would indicate that, in our preparation, the antibody primarily recognises AβPs, since the increased immunoreactivity which was observed following chronic hypoxia was fully prevented by inhibition of γ secretase as well as β secretase (Fig. 3). Similarly, the functional consequence, i.e. increased functional expression of L-type Ca^{2+} channel (Fig. 4) was also prevented by either secretase inhibitor (Fig. 4). Importantly, the use of these inhibitors provides compelling evidence that formation of AβPs is a requisite step mediating the effects of hypoxia on L-type Ca^{2+} channels. As such, these findings provide a direct mechanistic clue to account for the increased incidence of AD in individuals who have previously experienced prolonged hypoxia.

The effect of hypoxia to up-regulate L-type Ca^{2+} channels in cerebellar granule neurons appeared selective, since blockade of L-type channels with nimodipine prevented any effects of hypoxia on whole-cell currents, and inhibition of N-type channels with ω-CgTx, which increases the proportion of total current attributable to L-type channels, exaggerated the effects of hypoxia. We did not attempt to inhibit P/Q type channels with ω-agatoxin GVIA, since in this preparation the toxin is not particularly selective [22]. Our evidence that the amyloid-mediated effect of hypoxia is selective in its action on L-type channels is also supported by a previous (albeit indirect) report suggesting that direct exposure to amyloid peptides up-regulates L-type channels in cortical neurons [4]. In contrast to PC12 cells, hypoxia did not induce a Cd^{2+} insensitive Ca^{2+} influx pathway in cerebellar granule neurones. This is consistent with our previous findings that

exogenous AβPs do not induce such a pathway in these or other central neurons [23,24].

In the CNS, L-type Ca^{2+} channels play key roles in a diverse range of functions [10], including synaptic plasticity/long term potentiation [2,38], memory and mood [2,34], and "excitation–transcription coupling" [3]. Thus, alterations in their functional expression caused by hypoxia are likely to have important and far-reaching effects. Perhaps more importantly, it may be through modulation of L-type Ca^{2+} channel expression that AβPs exert a major neurodegenerative effect. There is a wealth of evidence to suggest that AβPs disrupt Ca^{2+} homeostasis as part of their toxic effect and whilst disruption of Ca^{2+} stores has received much attention as a target system for disruption in AD [17,19], there is compelling evidence that pharmacological blockade of L-type Ca^{2+} channels offers protection against the toxicity of AβPs [5,35,36] and that AβPs can up-regulate Ca^{2+} currents in central neurons [23,24].

In summary, our results demonstrate that prolonged hypoxia selectively up-regulates the functional expression of L-type Ca^{2+} channels in cerebellar granule neurones. This effect is dependent on the increased production of amyloid peptides of Alzheimer's disease. Given the central role of these channels in neuronal Ca^{2+} homeostasis and their involvement in at least some of the toxic effects of amyloid peptides, these results offer a cellular basis for understanding why prolonged hypoxia increases the likelihood of developing Alzheimer's disease.

References

[1] Annaert W, De Strooper B. A cell biological perspective on Alzheimer's disease. Annu Rev Cell Dev Biol 2002;18:25–51.

[2] Bauer EP, Schafe GE, LeDoux JE. NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. J Neurosci 2002;22:5239–49.

[3] Deisseroth K, Mermelstein PG, Xia H, Tsien RW. Signaling from synapse to nucleus: the logic behind the mechanisms. Curr Opin Neurobiol 2003;13:354–65.

[4] Ekinci FJ, Malik KU, Shea TB. Activation of the L voltage-sensitive calcium channel by mitogen-activated protein (MAP) kinase following exposure of neuronal cells to beta-amyloid, MAP kinase mediates beta-amyloid-induced neurodegeneration. J Biol Chem 1999;274:30322–7.

[5] Freir DB, Costello DA, Herron CE. A beta 25-35-induced depression of long-term potentiation in area CA1 in vivo and in vitro is attenuated by verapamil. J Neurophysiol 2003;89:3061–9.

[6] Green KN, Boyle JP, Peers C. Hypoxia potentiates exocytosis and Ca^{2+} channels in PC12 cells via increased amyloid β peptide formation and ROS generation. J Physiol 2002;541:1013–23.

[7] Green KN, Peers C. Amyloid beta peptides mediate hypoxic augmentation of Ca^{2+} channels. J Neurochem 2001;77:953–6.

[8] Green KN, Peers C. Divergent pathways account for two distinct effects of amyloid b peptides on exocytosis and Ca^{2+} currents: involvment of ROS and NFκB. J Neurochem 2002;81:1043–51.

[9] Held B, Pocock JM, Pearson HA. Endothelin-1 inhibits voltage-sensitive Ca^{2+} channels in cultured rat cerebellar granule neurones via the ET-A receptor. Pflugers Arch 1998;436:766–75.

[10] Hell JW, Westenbroek RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, et al. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel alpha 1 subunits. J Cell Biol 1993;123:949–62.

[11] Incalzi RA, Gemma A, Marra C, Muzzolon R, Capparella O, Carbonin P. Chronic obstructive pulmonary disease. An original model of cognitive decline. Am Rev Respir Dis 1993;148:418–24.

[12] Jendroska K, Hoffmann OM, Patt S. Amyloid B peptide and precursor protein (APP) in mild and severe brain ischemia. Ann NY Acad Sci 1997;826:401–5.

[13] Johnson-Wood K, Lee M, Motter R, Hu K, Gordon G, Barbour R, et al. Amyloid precursor protein processing and AB42 deposition in a transgenic mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 1997;94:1550–5.

[14] Kogure K, Kato H. Altered gene expression in cerebral ischemia. Stroke 1993;24:2121–7.

[15] Koistinaho J, Pyykonen I, Keinanen R, Hokfelt T. Expression of beta-amyloid precursor protein mRNAs following transient focal ischaemia. Neuroreport 1996;7:2727–31.

[16] Kokmen E, Whisnant JP, O'Fallon WM, Chu CP, Beard CM. Dementia after ischemic stroke: a population-based study in Rochester, Minnesota (1960–1984). Neurology 1996;46:154–9.

[17] LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci 2002;3:862–72.

[18] Mattson MP. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. Physiol Rev 1997;77:1081–132.

[19] Mattson MP, LaFerla FM, Chan SL, Leissring MA, Shepel PN, Geiger JD. Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. Trends Neurosci 2000;23:222–9.

[20] Moroney JT, Bagiella E, Desmond DW, Paik MC, Stern Y, Tatemichi TK. Risk factors for incident dementia after stroke. Role of hypoxic and ischemic disorders. Stroke 1996;27:1283–9.

[21] Moroney JT, Bagiella E, Desmond DW, Paik MC, Stern Y, Tatemichi TK. Cerebral hypoxia and ischemia in the pathogenesis of dementia after stroke. Ann NY Acad Sci 1997;826:433–6.

[22] Pearson HA, Sutton KG, Scott RH, Dolphin AC. Characterization of Ca^{2+} channel currents in cultured rat cerebellar granule neurones. J Physiol 1995;482:493–509.

[23] Price SA, Held B, Pearson HA. Amyloid beta protein increases Ca^{2+} currents in rat cerebellar granule neurones. Neuroreport 1998;9:539–45.

[24] Ramsden M, Henderson Z, Pearson HA. Modulation of Ca^{2+} channel currents in primary cultures of rat cortical neurones by amyloid beta protein (1–40) is dependent on solubility status. Brain Res 2002;956:254–61.

[25] Ramsden M, Plant LD, Webster NJ, Vaughan PF, Henderson Z, Pearson HA. Differential effects of unaggregated and aggregated amyloid beta protein (1–40) on K^+ channel currents in primary cultures of rat cerebellar granule and cortical neurones. J Neurochem 2001;79:699–712.

[26] Randall A, Tsien RW. Pharmacological dissection of multiple types of Ca2+ channel currents in rat cerebellar granule neurons. J Neurosci 1995;15:2995–3012.

[27] Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001;81:741–66.

[28] Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, et al. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 1999;402:537–40.

[29] Sinha S, Lieberburg I. Cellular mechanisms of beta-amyloid production and secretion. Proc Natl Acad Sci USA 1999;96:11049–53.

[30] Smith IF, Boyle JP, Green KN, Pearson HA, Peers C. Hypoxic remodeling of Ca^{2+} mobilization in type I cortical astrocytes: involvement of ROS and pro-amyloidogenic APP processing. J Neurochem 2004;88:869–77.

[31] Tatemichi TK, Paik M, Bagiella E, Desmond DW, Stern Y, Sano M, et al. Risk of dementia after stroke in a hospitalized

cohort: results of a longitudinal study. Neurology 1994;44:1885–91.

[32] Taylor SC, Batten TF, Peers C. Hypoxic enhancement of quantal catecholamine secretion. Evidence for the involvement of amyloid beta-peptides. J Biol Chem 1999;274:31217–22.

[33] Taylor SC, Peers C. Chronic hypoxia enhances the secretory response of rat phaeochromocytoma cells to acute hypoxia. J Physiol 1999;514:483–91.

[34] Veng LM, Mesches MH, Browning MD. Age-related working memory impairment is correlated with increases in the L-type calcium channel protein alpha1D (Cav1.3) in area CA1 of the hippocampus and both are ameliorated by chronic nimodipine treatment. Mol. Brain Res 2003;110:193–202.

[35] Yagami T, Ueda K, Asakura K, Kuroda T, Hata S, Sakaeda T, et al. Effects of endothelin B receptor agonists on amyloid beta protein (25–35)-induced neuronal cell death. Brain Res 2002;948:72–81.

[36] Yagami T, Ueda K, Sakaeda T, Itoh N, Sakaguchi G, Okamura N, et al. Protective effects of a selective L-type voltage-sensitive calcium channel blocker, S-312-d, on neuronal cell death. Biochem Pharmacol 2004;67:1153–65.

[37] Yokota M, Saido TC, Tani E, Yamaura I, Minami N. Cytotoxic fragment of amyloid precursor protein accumulates in hippocampus after global forebrain ischemia. J Cereb Blood Flow Metab 1996;16:1219–23.

[38] Zakharenko SS, Zablow L, Siegelbaum SA. Visualization of changes in presynaptic function during long-term synaptic plasticity. Nat Neurosci 2001;4:711–7.