Hypoxia and Alzheimer’s disease

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Abstract

Numerous cardiorespiratory disorders result in persistent systemic hypoxia, or at worst (as a consequence of stroke) deprive the brain of oxygen completely for a period of time. Patients suffering from such conditions are much more susceptible to the development of dementias such as AD (Alzheimer’s disease). Until recently, the cellular and molecular basis for the predisposition to AD by systemic hypoxia has been completely unknown. However, emerging evidence suggests that pathological cellular remodelling caused by chronic hypoxia shows striking similarities to those observed in the central nervous system as a consequence of AD. Furthermore, prolonged hypoxia can induce formation of Aβs (amyloid β peptides), the primary neurotoxic elements of AD, which accumulate over years to form the extracellular plaques that are the hallmark feature of the disease. Hypoxia can lead to paradoxical increases in mitochondrial ROS (reactive oxygen species) generation upstream of Aβ formation. The downstream consequences of prolonged hypoxia include remodelling of functional expression of voltage-gated calcium channels and disturbance of intracellular calcium homoeostasis via disrupted calcium buffering and inhibition of calcium extrusion mechanisms. These effects can be mimicked by application of exogenous...
Aβ and, crucially, appear to depend on Aβ formation. Current knowledge supports the concept that prevention of the deleterious effects of hypoxia may prove beneficial in slowing or preventing the onset of AD.

Introduction

Dementias, of which AD (Alzheimer’s disease) is by far the most common, are characterized by a progressive decline in memory, which becomes increasingly associated with confusion over simple, everyday tasks. As time passes, symptoms worsen and problems with memory become more obvious, as does confusion and general cognitive impairment. In later stages, mood swings, depression and anger are apparent and general mental deterioration can progress ultimately to death. Dementias are most commonly associated with old age; indeed, senile dementia specifically refers to patients over 65, and the risk of developing dementia doubles every 5 years after this age. The current global statistics surrounding dementia are alarming: worldwide there are an estimated 24–25 million people suffering from dementia. Each year, 4.6 million new cases arise (one every 7 s), which will lead to an estimated doubling of sufferers every 20 years [1]. This is a consequence of the global aging of the general population since there is no evidence that the rate at which AD presents in 65-year-olds and over is increasing. The preceding facts are in themselves shocking, but the impact on society is worsened by socioeconomic factors: the cost of caring for AD patients is greater than the combined cost of caring for cancer, stroke and heart disease patients. AD is clearly a major, worldwide current and future concern.

A brief scan through the clinical literature will reveal numerous reports which collectively suggest that diseases associated with a lowering of systemic oxygen levels predispose individuals to AD (reviewed briefly in [2]). The most striking, but by no means exclusive, data are from patients who have recovered from a stroke. The likelihood of such patients subsequently developing dementias is several-fold higher than it is for age-matched subjects who have not faced such an insult. One of the clearest accounts is the longitudinal study by Desmond et al. [3] who found the incidence of dementias increased approximately 6-fold in stroke patients (Figure 1). Importantly, they concluded that the risk of dementia amongst stroke patients was particularly high if associated with complications resulting in subsequent cerebral hypoxia or ischaemia. Despite this study, and others resulting in similar conclusions, no cellular or molecular basis for the association of hypoxia/ischaemia with AD had been forthcoming until approximately 5 years ago. This chapter will review the data which are accumulating to account for this association, but first commences with a brief account of the biochemical basis for AD, and current models for neuronal death which underlie this disease.

Cellular production of amyloid peptides

The hallmark feature of AD is the presence of extracellular plaques in the brain, along with intracellular neurofibrillary tangles (composed of
hyperphosphorylated tau protein, see [4] for recent review). Although tangles are clearly of great importance in the disease, most research has focused on the major constituent of the extracellular plaques, Aβ (amyloid β peptide), since most evidence suggests this peptide is responsible for neuronal death in AD. Aβ is a cleavage product formed by the sequential actions of two enzymes on APP (amyloid precursor protein). APP is a ubiquitously expressed, membrane-spanning protein which can undergo alternative cleavage pathways as illustrated in Figure 2. When APP is cleaved by α-secretase, this occurs within the Aβ sequence, precluding Aβ formation and instead yielding sAPPα, which is believed to be neuroprotective [5]. The identity (or identities) of the α-secretase remain to be fully clarified, but most evidence suggests it/they belong to the ADAM (A Disintegrin And Metalloprotease) family of proteases [6]. Subsequent cleavage of the α-secretase product yields additional fragments which may be biologically active [2]. The alternative amyloidogenic processing of APP involves the sequential activity of two enzymes, β- and γ-secretase (Figure 2). β-secretase, also known as BACE-1 (β-amyloid cleavage enzyme), Asp2 or memapsin2, is a single protein of the aspartyl protease family. By contrast, γ-secretase is a protein complex, consisting of presenilin (−1 or −2, where the cleavage site resides), nicastrin, Aph-1 and Pen-2 [7]. Both β- and γ-secretase present attractive therapeutic targets in AD, since both are required to liberate Aβ.

It is important to note that Aβ formation is a physiological process, and there is accumulating evidence that the peptide serves important physiological roles [2]. Levels of Aβ are maintained by its clearance and degradation, the latter
being a function of peptidases such as insulin-degrading enzyme, endothelin-converting enzyme and nephrilysin [8]. An imbalance between the production and the removal of Aβ is believed, according to the amyloid cascade hypothesis, to account for the development of neuronal dysfunction and, ultimately, death in AD. The stages of disease progression from initiation of amyloid burden to final deposition in plaques, and the consequences for neurons and other central nervous system cells during this period, currently form the basis of research interests of a large community of scientists whose common aim is to understand, and so prevent, this growing disease.

**Cellular dysfunction and death in AD**

The development of extracellular amyloid plaques and intraneuronal neurofibrillary tangles of hyperphosphorylated tau are well documented features of AD progression. So too is the loss of synaptic integrity and neuronal death in limbic and associated cortical regions of the brain. Less well understood, however, is the cellular and molecular basis for neuronal death. Clearly, evidence points towards Aβ causing apoptotic cell death, but the route of destruction
remains to be fully evaluated [9,10]. Nevertheless, a vast literature of studies addressing this issue has arisen over the past 15 years or so, and from such studies, some clear themes arise pointing to major pathways involved in cell death in AD. These are summarized in Figure 3. In brief, there are two fundamental mechanisms of primary importance in Aβ-induced neuronal death, one involving free radical/ROS (reactive oxygen species) generation, and the other centering on calcium dyshomoeostasis [9,11,12]. The whole process is complicated by the fact that these two pathways are inextricably linked at many levels. Thus, for example, increased ROS production can disrupt the functional expression of various types of ion channels, pumps and transporters, any of which can contribute to calcium homoeostasis. Disruption of calcium homoeostasis
(which can even occur due to the action of presenilin, independently of its ability to generate Aβ; [13]) can lead to mitochondrial dysfunction which can lead in turn to increased ROS formation. The two pathways ultimately lead to DNA condensation and fragmentation via caspase activation, but this is by no means the only point at which these enzymes act in this pathway [10]. Further complexity is added to the system by additional factors including the involvement of NF-κB (nuclear factor κB; which can be protective in neurones, but destructive via oxidative damage when activated in glia [14]) and a protective role for elevated calcium levels via PKB (protein kinase B)/Akt activation [15]. Different aspects of these pathways are championed by various laboratories, but in reality it seems we are some way yet from determining which mechanism is of primary importance, or whether both suggested pathways actually represent one complex mechanism for cell death in AD.

Hypoxic promotion of Aβ formation

Until recently, despite the clinical associations of hypoxia/ischaemia with AD, the cellular basis for such an association was unknown. However, the primary mechanisms of cellular dysfunction associated with Aβ (increased ROS production and disruption of calcium signalling; Figure 3) share numerous commonalities with the effects of hypoxia, prompting an examination into mechanisms by which hypoxia and AD may be linked at the cellular and molecular level. Much of our own group’s efforts have been aimed at understanding this linkage, and the fundamental observation which we have made is that exposing cells in tissue culture to periods of hypoxia (1–2.5% oxygen for 6–48 h) increases the production of amyloid peptides. We have demonstrated this in a variety of cell types, including PC12 cells, HEK (human embryonic kidney)-293 cells and the human neuroblastoma, SH-SY5Y [16–18], as well as in primary cultures of cortical astrocytes [19,20] and, importantly, central neurones [21]. Thus this common feature seems to occur regardless of cell type. However, the mechanism by which this occurs does appear cell-specific. For example, in SH-SY5Y cells, hypoxia promotes suppression of ADAM10 (a candidate α-secretase) expression and decreases sAPPα secretion [22], but in cortical astrocytes hypoxia is pro-amyloidogenic via increased expression of presenilin-1, a key component of the γ-secretase complex [20]. Our knowledge of how hypoxia promotes amyloidogenic APP processing is still in its infancy, but the consequences of such effects have been studied in more depth, shedding more light on the mechanistic links between the damaging effects of hypoxia and the neurodegeneration of AD. Of these, we shall focus on two key aspects of pathological remodelling of cell function; (i) calcium channel expression and (ii) calcium signalling pathways.

Remodelling of calcium channel functional expression

Early studies demonstrated that chronic (24 h) hypoxia, in addition to promoting Aβ formation (see above), also potentiated depolarization-evoked,
calcium-dependent neurotransmitter release, due to augmentation of calcium influx [16,23]. In PC12 cells, this was confirmed using patch-clamp recordings: hypoxia augmented the whole-cell calcium current, an effect entirely due to selective, functional up-regulation of L-type (Cav1) calcium channels, despite the presence in these cells of other voltage-activated calcium channels [24]. Importantly, this effect has also been demonstrated in primary cultures of central neurones [21]. Furthermore, hypoxic increases in whole cell calcium channel currents were also observed in HEK-293 cells stably expressing the α_{1C} subunit of the human L-type calcium channel, Cav1.2 [17]. Use of a recombinant expression system permitted detailed examination of the underlying mechanisms, which are summarized in Figure 4. Immediately, the reproduction of the effects of hypoxia in a recombinant expression system makes

**Figure 4. Hypoxic modulation of calcium channel expression**
In normoxia (A) calcium channel synthesis, trafficking and membrane insertion are depicted. This process is disrupted in hypoxia (B) due to increased ROS production and Aβ formation. Evidence points to a possible direct interaction of Aβ with the calcium channel protein, disrupting trafficking via an unknown mechanism which results in more active channels present in the plasma membrane. PS-1, presenilin-1.
the possibility that hypoxia increases calcium channel transcription highly unlikely. Instead, we observed an increase in post-transcriptional trafficking of L-type channels such that at any given time more were present and active in the plasma membrane, even though total cell channel content was not markedly altered. Crucially, these effects of hypoxia were inhibited by selective inhibitors of either β- or γ-secretase, indicating an absolute requirement for Aβ in this process [17]. Indeed, the altered trafficking may have resulted from a close or even direct interaction of Aβ with the calcium channel subunit, since the two proteins co-localized and co-immunoprecipitated [17].

The above-described process provides an attractive mechanism by which hypoxia could contribute to cell death in AD via disruption of calcium homoeostasis, as outlined in Figure 2. There is also evidence for the involvement of ROS production in this phenomenon, since antioxidants prevented hypoxic up-regulation of calcium channels in the same recombinant expression system [18]. Perhaps surprisingly, ROS involvement in this mechanism appears to be upstream of Aβ formation and the ROS appeared to be derived from the mitochondrial respiratory chain, since effects were inhibited by rotenone (an electron chain transport inhibitor) and by functional mitochondrial depletion induced by sustained exposure to ethidium bromide (cells derived this way are termed ρ0 cells). Furthermore, exposure of ρ0 cells to oxidative stress, or to Aβ itself, reproduced the effects of hypoxia to increase calcium channel up-regulation [18]. Increased ROS production from mitochondria has long been associated with aging, and appears to be intimately involved with the progression of AD [25]. Furthermore, there is a growing body of evidence to suggest ROS production from mitochondria paradoxically increases during hypoxia [26]. Collectively, these data provide an attractive scheme to account for a mechanism by which hypoxia could contribute to both calcium- and ROS-mediated neuronal damage or death in AD, via disruption of calcium channel functional expression.

**Hypoxic disruption of calcium signalling**

Although voltage-gated calcium entry is of paramount importance in neurons, other mechanisms for regulating calcium influx and, indeed, intracellular calcium buffering are equally important. Indeed, in non-excitable cells such as astrocytes, voltage-gated calcium entry has little or no physiological role, and calcium signalling proceeds by entirely different mechanisms. Agonists binding to phospholipase C-coupled receptors generate IP3 (inositol trisphosphate) which can mobilize calcium from the ER (endoplasmic reticulum) which in turn activates capacitative- (or store-operated) calcium entry. These means of calcium signalling are fundamental to numerous functions in the central nervous system and other tissues [27,28]. Exposure of various cell types to chronic hypoxia caused apparent augmentation of agonist-evoked mobilization of calcium from ER stores and additionally modulates capacitative calcium entry [29–31]. This has been studied in depth in primary cultures of astrocytes, cells...
which rely heavily for many functions on intra- and inter-cellular calcium wave propagation [32]. These effects are summarized in Figure 5. In essence, prolonged hypoxia augments agonist-evoked rises of [calcium], not by increasing the ER calcium content, but by inhibiting clearance of calcium from the cytosol, thereby causing its accumulation. Inhibition arose in part from suppressed expression of the plasmalemmal Na⁺/Ca²⁺ exchanger [33], but also from calcium-loading of mitochondria. This latter effect, in turn, appeared to be due to hyperpolarization of the mitochondrial membrane potential upon which

![Figure 5. Hypoxic modulation of calcium signalling](image)

**Figure 5. Hypoxic modulation of calcium signalling**

Schematic indicating agonist-mediated alterations in [calcium], in normoxia (A) and in chronic hypoxia (B). In both cases, agonist application liberates calcium from the ER, which is normally buffered by mechanisms including extrusion on the Na⁺/Ca²⁺ exchanger and uptake into mitochondria (A). Following chronic hypoxia (B), these two buffering mechanisms are prevented, leading to larger rises of [calcium], when cells are exposed to the agonist. The mechanism of Na⁺/Ca²⁺ exchanger inhibition is unknown, but the ability of mitochondria to buffer rises of [Ca²⁺] is due to the fact that chronic hypoxia has already caused them to become overloaded with calcium through hyperpolarization [30]. These damaging changes are associated with increased ROS levels derived from mitochondria, and increased Aβ production, presumably as a result of increased levels of the pro-amyloidogenic presenilin-1 (PS-1).
calcium uptake relies [31]. Again, these effects were seen to be associated with, indeed reliant upon, increased Aβ formation and generation of ROS [20]. Thus, as with remodelling of calcium channel functional expression, perturbations in calcium homeostasis and signalling caused by hypoxia involves pro-amyloidogenic processes.

Conclusions

It is clear from numerous clinical studies that conditions which disrupt oxygen delivery to the central nervous system (of which stroke is the most severe and well-documented example) promote the onset of dementias in general, and AD in particular. At the cellular level, restriction of oxygen supply promotes formation of ROS from mitochondria and disrupts the functional expression of numerous proteins associated with calcium homeostasis, including ion channels and transporters. Strikingly, ROS production and calcium dyshomoeostasis are two recognized pathways leading to neuronal death in AD and, indeed, hypoxia also promotes formation of Aβ. These cellular studies provide potential mechanisms to account for the association of hypoxic/ischaemic episodes and increased incidence of AD. In so doing, they provide clues as to targets which might prove useful in ameliorating the long-term consequences of such episodes.

Summary

• AD is a neurodegenerative disease in which neuronal death occurs via complex pathways involving generation of ROS and disturbance of intracellular calcium homeostasis.
• Hypoxic or ischaemic events, such as stroke, dramatically increase the likelihood of developing AD.
• Hypoxia promotes the formation of Aβ, the primary neurotoxic element of AD.
• Many of the adverse effects of prolonged hypoxia on cellular processes crucial to intracellular calcium homeostasis are mediated by increased ROS production and require Aβ formation.
• Current data suggest that oxygen availability may be a major determinant of Aβ formation and that mechanisms contributing to hypoxic/ischaemic cellular damage and death are similar to those of AD.

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References


