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Vascular Hypoperfusion, Mitochondria Failure and Oxidative Stress in Alzheimer Disease

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Alzheimer disease and cerebrovascular accidents (CVA) are the two leading causes of age-related dementia. Increasing evidence supports the notion that the underlying mechanism responsible for CVAs is also responsible for age-related dementia. The pathogenesis, which is primarily responsible for both disease processes, seems to involve chronic hypoperfusion. Hypoperfusion appears to induce chronic oxidative damage in tissues and cells, largely due to the generation of reactive oxygen and reactive nitrogen species (ROS and RNS respectively). Any condition that outpaces the capacity of endogenous redox systems to neutralize these toxic intermediates results in an imbalance. This redox imbalance is generally referred to as the *"oxidative stress"* and is associated with other age-related degenerative disorders, such as atherosclerosis, ischemia/reperfusion, and rheumatic disorders. Moreover, this chronic injury stimulus can also induce hypoperfusion in the microcirculation of vulnerable brain regions. Ultimately, hypoperfusion leads to energy failure, which manifests itself as damage to mitochondrial ultrastructure, in the formation of a large number of non-mature, or so-called *"young"*, electron dense *"hypoxic"* mitochondria and by overproliferation of abnormal mitochondrial DNA (mtDNA). Additionally, these mitochondrial abnormalities are found to coexist with increased redox metal activity, overexpression of lipid peroxidation markers and with RNA oxidation. This oxidative stress occurs within various cellular compartments, most notably in the vascular endothelial cell (EC), responsible for atherosclerotic damage. Nevertheless, vulnerable neurons, and associated glial cells, show mtDNA deletions and the overexpression of oxidative stress markers only in regions proximal to the damaged vessels. This evidence strongly indicates that it is the chronic hypoperfusion that induces lesions and causes the accumulation of the oxidative stress products. Therefore, any pharmacological intervention, directed at correcting the chronic hypoperfusion state, would possibly change the natural course of development of dementing neurodegeneration.

Key Words: Alzheimer disease, Vascular factors, Oxidative stress, Free radicals, Mitochondria, Metabolism, Neurodegeneration.

Introduction

The vascular endothelium, neurons and glia are all able to synthesize, store and release reactive oxygen species (ROS) and vasoactive substances in response to certain stimuli, especially those by chronic hypoxia/hypoperfusion, and their contribution to the pathophysiology of stroke, cerebrovascular disease or cerebrovascular accidents (CVAs) and Alzheimer disease (AD) is extremely important. The role of hypoperfusion, as a key factor for vascular lesions that causes oxidative stress, appears to be widely accepted as an initiator of AD (Aliev 2002, de la Torre 2002a). This idea is based on a positive correlation between AD

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and cardiovascular diseases. ROS are generated at sites of injury and/or inflammation. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissue, is a major target of oxidant stress, playing a critical role in the pathophysiology of several vascular diseases. Specifically, accumulated oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesions, followed by alterations in endothelial signal transduction and redox-regulated transcription factors. We hypothesize that the cellular and molecular mechanisms by which hypoperfusion-induced ROS-accumulation impairs endothelial barrier function and promotes leukocyte adhesion, induces alterations in normal vascular function and results in the development of AD. The sustained hypoperfusion and then oxidative stress of brain tissues could also stimulate the secondary damage via the overexpression of inducible and neuronal specific nitric oxide synthase (NOS: iNOS and nNOS, respectively) and endothelin-1 (ET-1) in brain cells.

There are many common underlying risk factors that play key roles in the development of cardiovascular, cerebrovascular and neurodegenerative diseases [for review and references see (Aliev et al. 2002a, de la Torre 2002a)]. For example, it has been widely accepted that tobacco smoking appears to be a risk factor for the development of cardiovascular, cerebrovascular, pulmonary diseases and cancer. In addition, cigarette smoking causes chronic hypoxic conditions, causing the formation of a large amount of free oxygen radicals that appear to be key factor in the development of these diseases. In support of this notion, new evidence has indicated that a continuous formation of free oxygen radicals induces cellular damage and decreases antioxidant defences. Several recent studies have shown that cigarette smoking is a cofactor in the initiation of AD via its effect on the vasculature (more discussion later). Vascular insufficiency/hypoperfusion are considered as a pathogenetic factor in the development of AD, and the positive relationship between cerebrovascular diseases such as stroke and especially cerebrovascular atherosclerosis indicates the latter may also be linked to the pathogenesis of AD. It is possible that continuous accumulation of oxidative stress products, such as peroxynitrite accumulation (via the overexpression of the iNOS and/or nNOS), appear to be secondary and accelerating factors for damage and for compromising the blood brain barrier (BBB) in hypoxia/hypoperfusion or AD.

In this review we outline recent evidence, as well as our own data, which indicates that a chronic injury stimulus induces the hypoperfusion seen in the microcirculation of vulnerable brain regions. Determining the mechanisms behind these imbalances may provide crucial information in the development of new, more effective therapies for the treatment of cerebrovascular diseases, including AD.

The Risk Factors for Alzheimer Disease and Stroke

Hypoxia/Ischemia/Reperfusion as Cofactors for Oxidative Stress-induced Cerebrovascular Lesions and Their Relationship to AD

The risk for Alzheimer dementia and stroke are known to increase at comparable rates with age. Recent advances suggest that vascular risk factors linked to cerebrovascular disease and stroke in the elderly significantly increase this risk (Kalaria 2000). Although some vascular lesions such as cerebral amyloid angiopathy, endothelial degeneration, and periventricular white matter lesions are evident in most AD cases, a third will exhibit cerebral infarction. Despite the interpretation of pathological evidence, longitudinal clinical studies suggest that stroke and AD occur in tandem more often than by chance alone (Hofman et al. 1997). Strokes often occur in patients with AD and have been linked to the pathogenesis of dementia (Kalaria 2000). Nevertheless, the nature of this relationship remains little explored. Is it possible that cerebral ischemia is a causal factor for AD? Irrespective of the ultimate pathogenic mechanism, these findings suggest that managing vascular disease is important in the treatment and prevention of AD (Aliev 2002, de la Torre 2002a) or mixed dementia (Kalaria 2000). Chronic hypoxia can alter cerebral microvessels ultrastructure, but this effect is heterogeneous and in some cases capillaries can respond to hypoxia independent of the arteriole (Weinbrecht et al. 1987). Animals exposed to three weeks of hypobaric hypoxia showed increased capillary density due to

the key role played by capillary segment elongation in the deeper layers of the cerebral cortex (Mironov et al. 1994). Therefore, prolonged hypoxia results in structural and functional adaptive responses that improve tissue oxygen delivery (Chavez et al. 2000). Other structural changes within brain microvessels include vascular proliferation and elongation (Stewart et al. 1997). Mitochondria of brain capillary EC maintain normal density in hypoxia, but the amount of mitochondria in the surrounding neuropil decreases significantly (about 30%) (Stewart et al. 1997). Moreover, rats exposed to hypobaric hypoxia showed increased basic fibroblastic growth factor (bFGF) mRNA in brain tissue (LaManna et al. 1994). During moderate hypobaric hypoxia, increased brain vasculature is associated with increased density of the brain capillary glucose transporter (Glut-1). However, this change is reversible and dependent on hypoxia exposure time (Harik et al. 1996). The same pattern has been observed in the microvascular system of the human AD brain (Aliev et al. 2000a, 2001, 2002, Shi et al. 2002a). Based on these findings, the relationship between oxidative stress markers and extracellular matrix binding ligands in the hypoxic brain during stroke and AD deserves further study. In addition, the injury induced by reperfusion after chronic hypoxia requires special attention, because the oxidative products that accumulates during hypoxia probably induces more tissue- and cellular-damage than the hypoxia itself.

Ischemia/reperfusion is a systemic process affecting the whole organ or tissue. Different types of blood cells have been involved in the pathogenesis of ischemia/reperfusion, including platelets, monocytes, neutrophils and others (Cirillo et al. 1994). Bednar et al. (1991) showed that neutrophils might be important contributors to ischemia-induced brain injury, whereas the role of platelets is more nebulous. In fact, systemic depletion of neutrophils reduced the volume of cerebral infarct after transient middle cerebral artery occlusion in the rat (Chen et al. 1992). EC affected by ischemia react in different ways. In the early stages injury is completely reversible upon reperfusion, but eventually tissue injury induced by ischemia passes a ***"point of no-return"*** and the damage becomes irreversible (Olah et al. 2001).

In the early period of ischemia the cells try to increase their surface available for gas and nutrient exchange by expressing cytoplasmic microvilli (Cirillo et al. 1992,1994, Aliev et al. 1993a, Salvatico et al. 1994, Sala et al. 1996) or by extending membrane protrusions into the vessel lumen (Dietrich et al. 1984, Cirillo et al. 1992, 1994, Aliev et al. 1993a, Salvatico et al. 1994, Sala et al. 1996). The appearance of these microvascular changes is related to the duration of ischemia and may be an adaptive EC response to altered hemodynamic conditions (Dietrich et al. 1984, Aliev et al. 1993a). The functional significance of microvilli, microblebs and other morphological changes is not clear, but they may have a role in the production of delayed, post-ischemic hypoperfusion by increasing vascular resistance (Dietrich et al. 1984, Aliev et al. 1993a).

The extent of EC injury mainly depends on the duration of ischemia and on the metabolic needs of the vascular system affected by ischemia. Also, the duration of experimental ischemia or acute anoxia required to cause damage varies for different organs. It takes approximately 10-15 min for irreversible damage to occur in brain (Fischer and Ames 1972, Wade et al. 1975, Dietrich et al. 1984). After long-term ischemia and the following reperfusion, the decreased number of active capillary vessels was associated with ultrastructural lesions in ischemic vessels and underlying tissues and cells (Cirillo et al. 1992, 1994, Aliev et al. 1993a, Salvatico et al. 1994, Sala et al. 1996). Cada and colleagues (Cada et al. 2000) demonstrated that decreased cerebral blood flow (CBF) in aging rats produces deficits in visuospatial behaviour after permanent surgical occlusion of both carotid arteries and that this deficit was correlated with metabolic abnormalities of the brain visualized by quantitative cytochrome oxidase histochemical mapping. These results suggest that deficits in visuospatial learning are not exclusively the result of hippocampal dysfunction, but may be directly correlated with altered oxidative energy metabolism in other integrative visuomotor regions identified in this study. They also suggest that chronic cerebrovascular ischemia in this aged rat model produces neurometabolic and behavioural alterations that may be relevant risk factors for the development of AD as well as other cerebrovascular accidents.

The Relationship between Tobacco Smoking and AD

It has been widely accepted that tobacco smoking causes cardiovascular, cerebrovascular, and pulmonary diseases and cancer through the damaging actions of free oxygen species. A growing body of evidence supports the idea that there is a positive correlation between AD and cardiovascular disease (Etiene et al. 1998, Kalaria 1999, 2000, Kalaria & Ballard 1999, de la Torre 2000a,b, Aliev et al. 2002, Aliev 2002). The epidemiological evidence indicates that tobacco smoking appeared to be a major risk for the development of human diseases including human AD via the production of ROS. However, it has been shown that nicotine is able to protect the neurons from injury stimuli (for details see later paragraph of this subsection). This may explain a part of the beneficial and protective effects of nicotine in a few neurodegenerative diseases, as suggested by many epidemiological studies [for review and references see Cormier et al. 2001]. Interactions between abnormal amyloid beta precursor protein (AβPP) metabolism and cholinergic dysfunction are increasingly apparent during AD. These major features of the disease both occur in restricted loci during normal aging, a potential model for early Alzheimer's type pathology. Particular attention has been given to the nicotinic acetylcholine receptors during the development of disease conditions (Court et al. 2000, Hellstrom-Lindahl & Court 2000). Previous observations have shown changes in the number of high-affinity nicotine binding sites in specific regions of the human brain during aging, and age- associated neurodegenerative diseases, including AD (Terzano et al. 1998). Nicotinic acetylcholine receptors (nAChR) are a class of ligand-gated channels composed of alpha and beta subunits with specific structural, functional and pharmacological properties. They participate in the physiological and behavioural effects of acetylcholine and they mediate responses to nicotine [for review and references see Court et al. (1997, 2000)]. These receptors are associated with numerous transmitter systems and their expression is altered during development and aging as well as by diseases such as autism, schizophrenia, AD, Parkinson disease (PD) and Lewy body dementia (LBD) [for review and references see Court et al. (1997, 2000)].

Nicotinic receptors contain a number of different subunits that are highly expressed during early human development. Disorders believed to be associated with abnormal brain maturation involve deficits in both α4β2, in the case of autism, and α7 (and possibly α4β2) in the case of schizophrenia. In aging and age-related neurodegenerative disorders nAChR deficits are predominantly associated with α4-containing receptors, although some studies also indicate the involvement of the α3 and α7 subunits. While aging appears to be associated with reductions in subunit mRNA as well as protein expression, during AD only protein loss is apparent (Court et al. 1997, 2000). The entorhinal cortex is particularly vulnerable to beta-amyloidosis, and compared with other cortical areas, it is remarkable for its relatively high density of nicotinic (^{3}H-nicotine) receptor binding as compared to cholinergic or glutamate binding [for references see (Perry et al. 1996, Court et al. 2000,Hellstrom-Lindahl & Court 2000).

During aging (between 40 and 100 years) high affinity nicotine binding in the frontal cortex decreases analogous to the glutamate NMDA receptor binding ([^{3}H] MK801) (Hellstrom-Lindahl & Court 2000). In the hippocampal formation and the entorhinal cortex nicotine binding also declines with the age, but NMDA receptor binding remains unchanged (Hellstrom-Lindahl & Court 2000). This reduction may predispose the neo- and archi-cortex to the loss of nAChRs observed in age-associated neurodegenerative conditions. In contrast, no age-related loss of nAChR binding is observed in the thalamus. Only after the 7th decade is some loss observed in the striatum (Hellstrom-Lindahl & Court 2000). However, in AD, PD and LBD, deficits in nAChRs are observed in these areas and may be associated with specific disease-related processes (Hellstrom-Lindahl & Court 2000). Nevertheless, a negative correlation between the α-3 mRNA density and age was observed in the entorhinal cortex of both Alzheimer's and normal subjects, suggesting a potentially extensive decay in α-3 -expressing neurons or loss of α-3 -containing receptors in intact neurons of the entorhinal cortex (Terzano et al. 1998).

With increasing age, post-maturity, there is a persistent decline in this nicotinic receptor binding in the entorhinal cortex. However, muscarinic M1 and non-M1, glutamate NMDA and non-NMDA receptors are spared (Perry et al. 1996). Normal elderly individuals, distinguished by the absence of beta A4 immunoreactive plaques in this area, are differentiated from those with plaques by higher nicotine binding (Perry et al. 1996). Individuals with an established history of smoking tobacco possess elevated nicotinic receptor binding and hippocampal choline acetyltransferase compared with non-smokers, and these individuals have a reduced density of cortical plaques (Perry et al. 1996). These findings are consistent with the hypothesis that down regulation of the nicotinic cholinergic receptor-ligands gated calcium channel controls the expression of neurotrophin, a chemical that plays a role in the evolution of Alzheimer-type pathology (Perry et al. 1996). In addition, the role of nicotinic receptors in AD as a potential therapeutic target has been considered recently (Martin-Ruiz et al. 1999).

Our hypothesis that cigarette smoking probably plays as a pathogenic cofactor in the initiation of AD via their effect on the vasculature. However, the role of tobacco smoking as a hypoperfusion and oxidative stress factor that may act as a pathogenic factor in the development of cerebrovascular and neuronal lesions in AD has not yet been given proper attention. Detailed ultrastructural studies elucidating the mechanisms behind the development of Aβ depositions, along with investigations into the possible accelerating effects of chronic hypoxia in an animal model as an example the effect of chronic hypoperfusion factors will likely open new avenues of treatment for Alzheimer patients. Therefore, more research is needed to determine the exact nature of this role.

Hypoperfusion as a Key Factor for the Reduction of Tissue Oxygen Delivery that Induces Oxidative Stress and Causes the Development of AD

One of main effects of chronic hypoperfusion induced vascular abnormality in AD appeared to be tissue oxygen deficiency. Recent several evidence indicate that chronic cerebral hypoperfusion induces reduction of tissue oxygen delivery that cost for the development of cognitive impairments such as AD (Kumar et al. 1990, Friston & Frackowiak 1991, De Jong et al. 1997, de la Torre 1997, 2002a). However, recent evidence reveals that a greater fraction of oxygen is removed from the vasculature in AD patients as compared to non-AD controls (Galle et al. 1995). This suggests that low vascular blood flow is a prominent feature of the brain during chronic hypoxia/hypoperfusion and may be a prime initiating factor during the development and maturation of AD (Meguro et al. 1999, de la Torre 2000a). It has been well recognized that the AD brain is characterized by the impairment of energy metabolism (Beal 1995). Positron emission tomography (PET) has revealed a decline in the cerebral metabolic rate of the parietal and temporal lobes during AD (Jagust 1988, Markesbery & Carney 1999). These metabolic defects are present before AD symptoms develop in Apolipoprotein E (ApoE) ε4 homozygote patients (Markesbery and Carney 1999). De la Torre (2000a) proposes that advanced aging with a comorbid condition, such as a vascular risk factor, which further decreases cerebral perfusion, promotes a *critically attained threshold of cerebral hypoperfusion (CATCH).* With time, CATCH induces brain capillary degeneration and suboptimal delivery of energy substrates to neuronal tissue (de la Torre 2000a). Because glucose is the main fuel of brain cells, its impaired delivery, together with a deficient delivery of oxygen, compromises neuronal stability because the supply for aerobic glycolysis fails to meet the brain tissue demand. The outcome of CATCH is a metabolic cascade that involves, among other things, mitochondrial dysfunction, oxidative stress, decreased adenosine triphosphate (ATP) production and increased calcium entry, abnormal protein synthesis, cell ionic pump deficiency, signal transduction defects, and neurotransmission failure. These events contribute to the progressive cognitive decline characteristic of patients with AD, as well as regional anatomic pathology, consisting of synaptic loss, SP, neurofibrillary tangles (NFT), tissue atrophy, and neuro-degeneration. CATCH identifies the clinical heterogenic pattern that characterizes AD because it provides compelling evidence that any of a multitude of different etiopathophysiologic vascular risk factors, in the presence of advanced

aging, can lead to AD (de la Torre, 2000a,b). We hypothesize that taken together with vascular EC and smooth muscle cell (SMC) abnormality, induced by hypoperfusion, is a key factor in the tissue oxygen delivery and therefore appeared to be main reason for the development of AD.

Effect of ROS on Brain Microvessels Function in AD

Vascular aging is associated with both structural and functional changes that can take place at the level of the endothelium, vascular smooth muscle cells (vSMC) and the extracellular matrix of blood vessels. In the endothelium, reduced vasodilatation on response to agonists occurs in large conduit arteries as well as in resistance arteries with the aging (Matz et al. 2000). Furthermore, enhanced oxidative stress by hypoperfusion contributes significantly to the deleterious effects of aging on the endothelium by means of NO breakdown due to ROS. The relative contribution of the above phenomenon to age-related endothelial dysfunction is highly dependent on the species and the type of vascular bed involved (Aliev et al. 1993a,b, Stewart-Lee et al. 1995, Aliev & Burnstock 1998, Matz et al. 2000).ROS are generated at sites of inflammation and injury, and at low levels, they can function as signalling intermediates in the regulation of fundamental cell activities such as growth and adaptation responses. At higher concentrations, ROS can cause cell injury and death. Specifically, oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesions, which are coupled with alterations in endothelial signal transduction and redox-regulated transcription factors (Lum and Roebuck 2001). We hypothesize that impair endothelial barrier function and promote leukocyte adhesion also induce alterations in normal vascular endothelial cell function, resulting in the development and the maturation of cerebrovascular and as well as AD. Because, compared to other organs or tissues, the brain is more vulnerable to ROS induced damage due to its high rate of oxygen consumption, high polyunsaturated lipid content, and relative paucity of classic antioxidant enzymes (Coyle and Puttfarcken 1993). In the AD brain there are increased regional levels of oxidative stress (Smith et al. 1996a, 1997a,b,1998, 2000, Perry et al. 2000). Recent studies have demonstrated a decline in polyunsaturated fatty acids (PUFA) (Prelli et al. 1988, Lovell et al. 1998, Prasad et al. 1998), increased levels of lipid peroxidation markers (Smith et al. 1996, Lovell et al. 1998), as well as protein oxidation (Markesbery 1997, Markesbery & Carney 1999), DNA oxidation (Mecocci et al. 1993, 1994, 1997) and RNA oxidation (Nunomura et al. 1999a,b, 2001, Aliev et al. 2002a) during AD. Additionally, the presence of oxidative stress markers such as advanced glycation end products (AGE), glycoxidative end products, e.g. N-ε-carboxy-methyl-lysine and lipid peroxidation adducts are detected in both neurofibrillary tangles (NFT) and senile plaques (SP) in AD (Smith et al. 1994, 1996a, 2000, Markesbery 1997, Sayre et al. 1997, Markesbery & Carney 1999, Perry et al. 2000) and in post-ischemic tissues (Granger et al. 1989, Cirillo et al. 1992, 1994, Salvatico et al. 1994, Sala et al. 1996).

Amyloid beta (Aβ) deposits, one of the prominent features of AD, are found in cortical and subcortical gray matter and in meningeal and gray matter blood vessels ***(congophilic angiopathy)*** (Kalaria 1999, 2000). *In vitro* experimental evidence has shown that these Aβ deposits induce cerebrovascular dysfunction in the rat brain (Price et al. 1997), and that the Aβ peptide produces endothelial dysfunction in cerebral microvessels via ROS. This occurs when the ROS scavenging enzyme (superoxide dismutase) prevents acetylcholine-induced endothelium-dependent vasodilation (Price et al. 1997). Moreover, now as well documented that the Aß peptide is responsible for the cerebrovascular effects of amyloid beta precursor protein (AßPP) overexpression (Iadecola et al. 1999, Niwa et al. 2000). Study by Iadecola and coworkers have demonstrated that transgenic mice overexpressing AßPP have a profound and selective impairment in endothelium-dependent regulation of the neocortical microcirculation. This indicates that peptides derived from APP processing may contribute to the alterations in cerebral blood flow (CBF) and neuronal dysfunction during AD (Iadecola et al. 1999). Amyloid beta 1-40 (Aß1-40) did not influence the CBF increasing produced by the endothelium-independent vasodilators and hypercapnia. In contrast, Aß1-42 did not attenuate resting CBF or the CBF increasing produced by

endothelium-dependent vasodilators. The superoxide scavengers SOD and MnTBAP reversed the cerebrovascular effects of Aß1-40. This data strongly suggests that Aß1-40, but not Aß1-42, produces the cerebrovascular alterations seen in AßPP transgenic, and thus, Aß1-40 could play a role in the cerebrovascular alterations observed in Alzheimer's dementia (Kalaria 2000, Niwa et al. 2000). Moreover, this study supports recent evidence that microvessels isolated from the AD brain kill neurons *in vitro* (Grammas et al. 1999). However, despite all the research on the effects of Aß, the source of the ROS *in vivo* and relation to hypoperfusion is not completely understood.

Pathological Hallmarks of Cerebrovascular Lesions and AD

The heterogeneous pathology of AD is due to variability in the nature and severity of vascular lesions and its co-existence with cerebrovascular diseases such as cerebrovascular arteriosclerosis (CVA) (Etiene et al. 1998). For example, there are significantly higher densities of Aβ immunopositive plaques in AD+CVA as compared to AD alone (Etiene et al. 1998). The Aβ deposits in senile plaque (SP) and cerebrovascular angiopathy are derived from AßPP expressed in neurons and in a variety of non-neuronal cells (some outside of the central nervous system) (Selkoe 1994, Octave 1995, Smith & Breslow 1997, Jakobovits et al. 2000, Hock and Lamb 2001, Kulnane and Lamb 2001). Several morphometric features of BBB dysfunction in patients with pathologically confirmed AD have been reported (Stewart et al. 1992). Accumulation of Aβ deposits around vessels in AD brain biopsy samples may be an indication of the breaking BBB during AD progression (Stewart et al. 1992, Aliev et al. 1999, 2002). Our recent finding (Aliev et al. 2002) strongly support the recent hypothesis that structural or functional abnormalities of the BBB itself may represent a seminal pathogenic event during AD, leading to vascular amyloid deposition in the brain (Prelli et al. 1988, Vinters et al. 1988, Stewart et al. 1992). Perivascular Aβ deposition may be a risk factor for reduced regional CBF (rCBF) (Crawford 1998). The age-related loss of mechanisms/cells that are capable of removing Aβ deposits involves subtle molecular alterations in the components of the basement membrane (BM) that bind Aβ and protect it from cellular degradation (Lindahl and Lindahl 1997), along with the activation of non-neuronal cells such as microglia which further contribute to neuronal damage (Kisilevsky 1998). Several factors that may ameliorate AD have either been associated with improved CBF or have prevented CBF decline (Crawford 1998). The vascular lesion in AD brain is associated with widespread penetration of Aβ deposits by degenerating microvessels (Kalaria 1999, 2000).

Numerous morphometric studies have demonstrated that endothelial cell (EC) contact with the vast majority of SP is by chance, and while it is not unusual to show a close proximity in the highly vascular brain (Kawai et al. 1990), there is an actual exclusion of vessels from most SP. It is also clear that a certain subpopulation of SP shows a real and intimate relationship with the vasculature (Kawai et al. 1990, Iwamoto et al. 1997). It is likely that SP have more than one origin (Perry et al. 1998, Perry and Smith 1998), and that vessels are one of their direct targets. In over 90% of AD cases, Aβ can be detected in at least some vessels (Joachim et al. 1989), and the source of this Aβ is likely vascular EC and SMC rather than neurons, since EC and SMC show abundant APP immunoreactivity (Coria et al. 1988, Kawai et al. 1990, Tagliavini et al. 1990, Aliev et al. 2002). Ultrastructural studies on blood vessels with Aβ deposits have shown their intermittent associations with membrane abnormalities of SMC (Perry et al. 1998, Perry and Smith 1998). Indeed, in AD cases with a clinical history of cerebral bleeding, the muscle layer is sometimes completely replaced by Aβ deposits (Coria et al. 1988, Kawai et al. 1990, Tagliavini et al. 1990). This finding suggests that vascular wall cell alterations such as EC damage and muscle cell atrophy may occur in AD, even in the absence of visible Aβ depositions, and indicates that vascular system appear to be a primary target for the development of this disease.

Relationships between APoE genotype, hypercholesterolemia and vascular changes in AD

The association of Aβ with cerebral vessels is an intriguing feature of AD. While some degree of cerebral Aβ angiopathy involving the leptomeninges and intraparenchymal vessels occurs

in almost all cases of AD, the proportion of microvessels within a neocortical region containing deposits of the Aβ peptide is not known (Thomas et al. 2000). In addition, the mechanisms behind the effects of several vascular factors and peripheral vascular pathophysiology might promote the late-onset of AD (Hofman et al. 1997, Kalaria and Ballard 1999, Skoog et al. 1999, Thomas et al. 2000). Apolipoprotein E (ApoE), a major risk factor for atherosclerosis (Aliev et al. 1993b, Smith & Breslow 1997, Aliev & Burnstock 1998), as well as AD (Roses 1995), may be linked to AD via its effects on the vasculature (Ellis et al. 1996, Etiene et al. 1998, Aliev 2002, de la Torre 2002a). Studies by Thomas and coworkers have demonstrated that in clinically and pathologically confirmed AD cases, the percentage of cerebral microvessels in the temporal cortex and parahippocampal gyrus is associated with the predominant Aβ1-42 form of the Aβ peptide (Thomas et al. 2000). Surprisingly, double immunostaining methods found that at least 40% of the microvessels in the two brain regions contained Aβ1-42 deposits (Thomas et al. 2000). However, there was no correlation of such localization with the ApoE genotype, although E4 homozygote revealed a greater Aβ1-40 burden. Observations suggest that high proportions of cortical microvessels are associated with Aβ1-42, which may affect microvascular function (Thomas et al. 2000). Moreover, the levels of total serum, low density lipoproteins (LDL), and ApoB are associated with increased deposition of Aβ in demented individuals with neuropathologically confirmed AD (Kuo et al. 1998). These findings indicate a key role for vascular abnormalities in the pathogenesis of AD. Since chronic hypoxia/hypoperfusion, Aβ depositions, and AD are maladies with similarities to atherosclerosis, we would expect them to share risk factors (Aliev et al. 2002, Aliev 2002, de la Torre 2002a). We would also expect that the same preventive interventions would alleviate their symptoms (Kuo et al. 1998, Aliev et al. 2002).

Hyperlipoproteinemia is associated with the impairment of NO-mediated, endothelium-dependent dilation (Galle et al. 1995). Galle and colleagues (Galle et al. 1995) demonstrated that oxidized lipoprotein (a) impairs endothelium-dependent dilation and is more potent than oxidized LDL in this effect. Accumulating evidence clearly indicates that comparisons between ventricular fluid (VF) lipoproteins isolated from AD patients and age-matched non-demented patients show that cerebrospinal fluid (CSF) lipoprotein metabolism is altered in AD (Montine et al. 1997). These data support our hypothesis that there is a direct relationship between vascular and lipoprotein abnormalities in AD. The positive linear relationship between AD and fat intake (Grant 1997, Smith et al. 1997) additionally relevant. A recent study (Refolo et al. 2000) showed direct evidence linking cholesterol metabolism and the development of AD in a transgenic mouse model. This work also indicates that diet-induced hypercholesterolemia resulted in significantly increased levels of formic acid-extractable Aβ peptides in the central nervous system (CNS) in AD mice. The total level of Aβ was strongly correlated with the level of cholesterol in both plasma and CNS. The Aβ level was also correlated with number and size of amyloid deposits (Refolo et al. 2000). These data demonstrate that dietary cholesterol increases Aβ accumulation and accelerates AD-related pathology in these animals. In addition, our recent finding demonstrated that the ultrastructural features of vascular lesions and mitochondria in brain vascular wall cells from human AD brain biopsy, human short post-mortem brain tissues, yeast artificial chromosome (YAC R140) and C57B6/SJL transgenic positive (Tg+) mice overexpressing amyloid beta precursor protein (AßPP) has same pattern (Aliev et al. 2002). *In situ* hybridization using mitochondrial DNA (mtDNA) probes for human wild type, 5kb deleted and mouse mtDNA and immunocytochemistry using antibodies against amyloid precursor protein (APP), 8-hydroxy-2'-guanosine (8OHG) and cytochrome c oxidase subunit 1 (COX) revealed similar pattern of their ultrastructural localization (Aliev et al. 2002). There was a higher degree of amyloid deposition in the vascular walls of the human AD, YAC and C57B6/SJL Tg (+) mice compared to aged-matched controls (Aliev et al. 2002). In addition, vessels with more severe lesions showed immunopositive staining for APP and possessed large, lipid-laden vacuoles in the cytoplasm of EC. Significantly more

mitochondria abnormalities were seen in human AD, YAC and C57B6/SJL Tg (+) mouse microvessels where lesions occurred (Aliev et al. 2002). *In situ* hybridization using wild and chimera (5 kB) mtDNA probes revealed positive signals in severely damaged mitochondria from the vascular endothelium and in perivascular cells of lesioned microvessels close to regions of large amyloid deposition. These features were absent in undamaged regions of human AD tissues, YAC and C57B6/SJL Tg (+) mouse tissues and in aged-matched control subjects. Especially, vessels with atherosclerotic lesions revealed endothelium and perivascular cells possessing clusters of wild and deleted mtDNA-containing positive probes (Aliev et al. 2002). These mtDNA deletions were accompanied by increased amounts of immunoreactive APP, 8OHG and COX in the same cellular compartment (Aliev et al. 2002). Our observations demonstrate that vascular wall cells, especially their mitochondria, appear to be a central target for oxidative stress induced damage before the development of AD pathology (Aliev et al. 2002). On the other hand, the positive correlation between AD and cholesterol levels suggests that antioxidant therapy and cholesterol lowering drugs could delay the occurrence of AD (Sparks 1997, Sparks et al. 2000). However, despite their frequencies, the pathophysiological and morphological changes in brain microcirculation that accompany AD remain poorly understood, and the specific factor controlling vascular tone in AD is unknown.

The Role of Mitochondria Abnormalities in the Pathogenesis of Oxidative Stress Induced Brain Lesions During the Development of AD

In aerobic cells 90-95% of the total amount of ATP production requires oxygen. The synthesis of ATP via the mitochondrial respiratory chain is the result of electron transport across the electron transport chain coupled to oxidative phosphorylation [for review and ref. see (Acuna-Castroviejo et al. 2001)]. Excitotoxicity, mitochondria dysfunction and free radical induced oxidative damage have all been implicated in the pathogenesis of several different neurodegenerative diseases, such as PD, amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) as well as AD. The main radical produced by mitochondria is the superoxide anion and the intramitochondrial antioxidant systems scavenge this radical to avoid oxidative damage, which can lead to impaired ATP production (Schulz et al. 1997, Fiskum et al. 1999, Castellani et al. 2002). Both processes, i.e., defective ATP production and increased oxygen radicals, may induce mitochondria-dependent cell death [for review and detail see (Schulz et al. 1997)]. During aging and some neurodegenerative diseases, including AD, damaged mitochondria are unable to maintain the energy demands of the cell (Hirai et al. 2001). This can lead to an increased production of free radicals, which induces the interruption of oxidative phosphorylation, resulting in decreased levels of ATP (Schulz et al. 1997).

Much of the interest in the association of neurodegeneration with mitochondrial dysfunction and oxidative damage emerged from animal studies using mitochondrial toxins (Schulz et al. 1997). These consequences have been strongly implicated in the pathogenesis of human as well as animal models of neurodegenerative diseases (Beckman & Ames, 1998a,b, 1999, Wallace 1999), particularly AD (Beal 1995, Bonilla et al. 1999, Fiskum et al. 1999, Aliev et al. 1999, 2002, Hirai et al. 2001, Castellani et al. 2002).

The effect of acute or chronic ischemia as well as neurodegenerative diseases on neuronal mitochondrial ultrastructure linearly correlate with the severity and the duration of the injury stimuli (Fiskum et al. 1999). It has been well documented that after long-term ischemia/reperfusion the mitochondria ultrastructure disintegrates *in vivo* and *in vitro* (Aliev et al. 1993a, Salvatico et al. 1994, Sala et al. 1996). Apoptosis of degenerating neurons occurs in association with the accumulation of perikaryal abnormal mitochondria and oxidative damage to the nucleus (Al Abdulla & Martin 1998). This same pattern of mitochondria lesions is observed in human AD brain biopsy samples (Aliev et al. 1999, Hirai et al. 2001). The reduced expression of both mtDNA and nuclear DNA encoded genes is consistent with a physiological down-regulation of the mitochondria respiratory chain in response to declining neuronal activity (Wallace 1997, 1999, Bonilla et al. 1999, Fiskum et al. 1999, Castellani et al. 2002). However, the role of somatic cells and mtDNA mutations in the pathogenesis of

mitochondria failure during AD is still controversial (Bonilla et al. 1999, Fiskum et al. 1999, Wallace 1999). Our recent findings indicate that mitochondria abnormalities appear to be key target in the development of AD-like pathology in YAC AßPP transgenic mice (Aliev et al. 2000a, 2001, 2002, Shi et al. 2002a). The deleted mtDNA is increased at least 3 fold in AD cases as compared to controls in humans (Hirai et al. 2001). Moreover, it has been reported that mitochondrial DNA isolated from the brains of AD patients shows oxidative modifications containing 8-hydroxy-2'-deoxyguanosine (8OHdG) (Mecocci et al. 1993, 1994, 1997). Additionally, studies using *in situ* markers for 8OHdG and 8-hydroxy-guanosine (8OHG) showed that RNA oxidation is a prominent feature of damaged neurons in AD (Nunomura et al. 1999a,b, 2001). Quantitative analysis revealed a strong positive correlation between mtDNA deletions and 8OHG (Hirai et al. 2001). However, given that mitochondrial DNA (even DNA containing the 5kb deletion) is spared relative to 8OHG, we suspect that mitochondrial abnormalities correlate, but do not directly produce ROS. Therefore, it is important to recognize that 8OHG is formed by the direct attack of •OH. These •OH molecules have only a 2nm sphere of diffusion and are unable to diffuse through the mitochondrial membrane (Hirai et al. 2001).

Recent observations by Cormier and coworkers have shown the effect of nicotine on rat brain mitochondria (Cormier et al. 2001). The polarographic studies determined the effects on the respiratory chain, whereas enzymatic assays and [H]-nicotine binding allowed them to precisely identify its target and site of action. The measurements of oxygen consumption showed a significant concentration-dependent inhibition by nicotine. Nicotine bound to complex I of the respiratory chain and inhibited the NADH-Ubiquinone reductase activity (Cormier et al. 2001). This study also showed that nicotine and NADH compete for complex I (Cormier et al. 2001). Effects of cotinine, the main nicotine metabolite, and nornicotine showed that nornicotine inhibit mitochondrial respiration whereas cotinine did not. Complex I generate the superoxide anion, and nicotine, following NBT (Nitobenzoyl toluene) T-bars assay oxidation, was able to inhibit this ROS generation (Cormier et al. 2001). However, more studies need to be done to determine the effect of nicotine on the mitochondria functions as well as DNA overexpression and/or deletion during the development of neurodegenerative disorders including AD. The exact cellular mechanisms behind vascular lesions and their relation to oxidative stress markers identified by RNA oxidation, lipid peroxidation, or mtDNA deletion remain unknown. Future studies comparing the spectrum of oxidative stress-induced damage during reperfusion injury or, more importantly, during hypoxia/hypoperfusion, with cognitive impairment such as AD damage are warranted.

The Potential Role of Endothelial Vasoactive Substances in the Pathophysiology of Brain Ischemia/Reperfusion and AD

The role of the EC in the control of vascular tone is mediated by the synthesis and release of vasoactive substances such as the endothelium-derived vasodilator NO (Moncada et al. 1991; Marletta, 1994; Moncada & Higgs 1995) and vasoconstrictor ET-1 (Yanagisawa et al. 1988). NO regulates vascular tone, platelet aggregation, leukocyte adhesion, SMC proliferation, synaptic neurotransmission and cytotoxic/cytostatic actions of macrophages (Moncada et al. 1991, Dawson et al. 1991, Dawson 1994, Dawson & Dawson 1996, Xia et al. 1996, Crow et al. 1997, Michel & Feron 1997, Stuehr 1997, Wever et al. 1998). This labile molecule may carry out important biological roles both within the cell in which it is synthesized, and by interacting with nearby cells and molecules (Ignarro 1990, Stamler 1994). Three distinct isoforms of NOS derived from different genes generate NO: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) (Moncada et al. 1991, Marletta 1994, Robinson et al. 1994, Sessa 1994, Moncada & Higgs 1995, Stuehr 1997). These isoforms are similar in structure and function (Marletta 1994, Sessa 1994, Stamler 1994, Stuehr 1997). eNOS was first purified and cloned from vascular endothelium, but has also been discovered in cardiac myocytes, blood platelets, brain cells [for references see: (Moncada et al. 1991, Morris & Billiar 1994, Sessa 1994,

Moncada & Higgs 1995, Xia et al. 1996, Michel & Feron 1997) and in cellular compartments such as mitochondria (Bates et al. 1995, 1996). The activity of eNOS is a major determinant of vascular tone and blood pressure, and is altered in diseases such as hypertension, diabetes, atherosclerosis, ischemia/reperfusion (Dawson 1994, Michel & Feron 1997, Wever et al. 1998, Aliev& Burnstock 1998, Aliev et al. 2000b) and AD (de la Torre et al. 2000b, Shi et al. 2002a).

A dynamic balance of relaxing and constricting factors regulates cerebrovascular tone. Constitutively produced NO normally influences basal cerebral vascular tone, and mediates vascular responses to diverse stimuli (Faraci 1991) and cerebral vasodilation (Faraci & Brian 1994). Impaired endothelium dependent relaxation of cerebral blood vessels has been observed during chronic hypertension, diabetes, hypercholesterolemia, subarachnoid haemorrhage (SAH), and ischemia (Faraci 1991, Faraci & Brian 1994, Aliev & Burnstock 1998). Several reports have shown that NO is also involved in regulating the cerebral circulation during hypercapnia (Iadecola 1992, Fabricius & Lauritzen 1994) and focal (Buisson et al. 1992, 1993, Dawson 1994, Samadani et al. 1997) or global brain ischemia (Hamada et al. 1994, Nakashima et al. 1995, Iadecola et al. 1995a,b, 1996). Iadecola (Iadecola 1992) demonstrated that arginine-derived NO mediates the powerful effects of CO_2 on cerebral circulation. NO synthesized by the action of nNOS participates in regulating basal CBF and is the major contributor to the hypercapnic CBF response (Wang et al. 1995). Chronic inhibition of constitutive NO production increases EC permeability during various vascular diseases (Wade et al. 1975, Moncada et al. 1991, Sessa 1994, Moncada and Higgs 1995, Aliev et al. 2000b, Aliev 2002). Due to its vascular effect, NO might improve tissue perfusion and exert a protective action. At the same time, overproduction, either by activation of nNOS by excitatory (Garthwaite & Beaumont 1989), or by induction of iNOS in glial, vascular or blood cells (Iadecola et al. 1995a,b, 1996) during the ischemic episodes, might be deleterious. Mice with the eNOS gene knock-out show that the NO synthesized by eNOS protects against ischemic damage by increasing CBF, whereas NO produced by nNOS contributes to lesions (Huang et al. 1994, Hara et al. 1996). The inhibition of NO synthesis by EC leads to increased intracellular oxidative stress, which induces neutrophil-EC interactions (Granger et al. 1989, Aliev et al. 1993a,b) and may promote the development and progression of vascular diseases such as atherosclerosis (Wever et al. 1998, Aliev & Burnstock 1998, Aliev et al. 2000b) and ischemia/reperfusion injury (Faraci 1991, Cirillo et al. 1992, 1994, Aliev et al. 1993a, Faraci & Brian 1994, Eliasson et al. 1997).

Expression of nNOS in the entorhinal cortex and hippocampus is affected in AD (Thorns et al. 1998). Dimethylargininase, primarily expressed in tissues containing the constitutive forms of NOS, like brain, kidney, and endothelium (Kimoto et al. 1993, 1995, Bogumil et al. 1998), regulates NO production by hydrolyzing free methylated arginine derivatives (effective endogenous inhibitors of NOS) (MacAllister et al. 1996). The expression of dimethylargininase is dramatically increased during AD (Smith et al. 1998). The presence of dimethylargininase abnormalities in the AD brain is not surprising since nitration, resulting from peroxynitrite or peroxynitrate, is increased in all neurons at risk of dying due to AD (Beckman, 1991, Beckman et al. 1993, Smith et al. 1997a). However, the ultrastructural localization of dimethylargininase immunoreactivity in different cellular compartments of the AD brain or in transgenic animal models of AD has yet to be described.

Excess NO production is found during excitotoxicity, inflammation and ischemia-reperfusion injury (Bredt et al. 1994), and the oxidation products of NO, namely peroxynitrite and peroxynitrate are powerful oxidants. Also, $ONOO^-$ can generate the highly reactive OH^0, a more powerful oxidant than NO itself (Bredt & Snyder 1994, Dawson & Dawson 1996, Smith et al. 1997a). The increased nitrotyrosine immunoreactivity in AD is found in the neuronal cytoplasm of the cerebral cortex within regions of neurodegeneration, yet it is undetectable in corresponding control regions (Smith et al. 1997a). This distribution is essentially identical to that of free carbonyls (Smith et al. 1996). The widespread occurrence of nitrotyrosine immunoreactivity in neurons

(Smith et al. 1997a) suggests that chronic oxidative damage is not restricted to long-lived polymers such as NFTs, but instead, reflects a generalized oxidative stress contributing to the pathogenesis of AD.

The expression of iNOS is found in a variety of cells in response to lipopolysaccharides, certain cytokines and ROS generators (Moncada et al. 1991, Dawson et al. 1991, 1994, Marletta 1994, Dawson 1994, Faraci & Brian 1994; Morris & Billiar 1994, Dawson & Dawson 1996, Michel & Feron 1997, Aliev 2002). Because iNOS produces much greater amounts of NO than either eNOS or nNOS (Ignarro 1990), it may be an important mediator of cytotoxicity in brain (Dawson et al. 1991). Recently, Iadecola and coworkers have proposed that iNOS makes a late contribution to ischemic brain damage (Iadecola et al. 1996). The catalytic activity of iNOS enzymes or mRNA expression were present in brain tissue after 2 hours of transient focal ischemia or 1-2 days after permanent focal ischemia (Iadecola et al. 1995a,b). It has been suggested that iNOS plays a role in the formation of NFT (Thorns et al. 1998).

NOS positive neurons are present in neuron subgroups throughout many regions of the brain (Dawson & Dawson 1996). Recent studies report that immunostaining for reduced NADPH-diaphorase, as well as nNOS and eNOS, reveals their presence in dendritic and axonal terminals that are closely associated with the middle cerebral artery and cerebral microvessels [for ref. see: (Dawson et al. 1991,1994, Dawson 1994, Dawson & Dawson 1996)]. The presence of L-arginine in astrocytes *in vivo* suggests that glia may store this chemical for NO production in brain (Dawson 1994, Dawson et al. 1994, Faraci & Brian 1994). Moreover, glial cells exhibit an inflammatory response during infection or ischemic disease. They also release pro-inflammatory cytokines and synthesize and release NO (Faraci & Brian 1994). The large amount of NO that is released from glial cells via the expression of iNOS after their stimulation is neurotoxic, because it induces oxidative stress, mitochondrial dysfunction and excitotoxicity (Dawson et al. 1994, Xia et al. 1996, Almeida et al. 1999). Hypoxic brain injury (acute or chronic) is associated with the formation of both NO (Beckman et al. 1990, 1994, Beckman 1991, Cazevieille et al. 1993, Faraci & Brian 1994, Dawson & Dawson 1996) and the superoxide anion, which may react to form free radicals (Xia et al. 1996, Smith et al. 1997a) and cause neurotoxicity (Beckman et al. 1990, Beckman 1991, Dawson et al. 1991, 1994, Radi et al. 1991, Lafon-Cazal et al. 1993a,b, Dawson & Dawson 1996, Xia et al. 1996). Investigations into determining the exact ultrastructural localization of the different NOS isoforms in the brain vascular tree, neurons and glia in post-hypoxic and AD brain would be warranted.

Endothelin-1 (ET-1) appears to be a vasodilator at physiologically relevant concentrations, and a potent vasoconstrictor in several pathologies associated with a rise in ET plasma and tissue levels (Yanagisawa et al. 1988, Lerman et al. 1990, 1992, Lerman and Burnett 1992, Brunner 1997). Several studies have shown that ET-1 immunoreactivity is increased in human atherosclerotic vessel wall cells (Lerman et al. 1990, 1992, Lerman & Burnett 1992, Aliev & Burnstock 1998, Aliev et al. 2000b), post-ischemic vascular lesions (Brunner 1997), aged rats (Aliev et al. 1995), and during other diseases such as metastatic adenocarsinoma of the prostate (Nelson et al. 1995) and human colorectal liver metastases (Shankar et al. 1998, Aliev et al. 2002b). ET-1 is not only produced by EC, but is also present in other cells in atherosclerotic tissues in humans and animals (Lerman et al. 1992, Aliev & Burnstock 1998, Aliev et al. 2000b). Increased expression of ET-1 immunoreactivity in thoracic aortic EC from human and animal models of atherosclerotic vessels (Aliev & Burnstock 1998, Aliev et al. 1998, 2000b, 2001) or after long-term sympathectomy (Aliev et al. 1996) is associated with the depression of eNOS immunoreactivity (Aliev & Burnstock 1998, Aliev et al. 1995, 1996, 1998, 2001, 2002b). An imbalance between endothelium-derived vasorelaxant and vasoconstrictor substances may play a key role in the development of chronic brain hypoxia and in the adaptive response of the brain to oxidative stress in ischemia and AD.

Transgenic Animals as Models for Studying Cerebrovascular and Neuronal Lesions in AD

Developing an animal model has been crucial for investigating the molecular and cellular etiology of AD (Lamb et al. 1997, 1999, Neve & Robakis 1998, Jakobovits et al. 2000, Hock & Lamb 2001, Kulnane & Lamb 2001, Kulnane et al. 2002). There are a

number of transgenic animals that overexpress normal AβPP or AβPP with familial AD (FAD) mutations or fragments of AßPP (Lamb et al. 1997, 1999, Neve & Robakis 1998, Jakobovits et al. 2000, Hock & Lamb 2001, Kulnane & Lamb 2001, Kulnane et al. 2002). During the past two decades it has become evident that clinical and neuropathological phenotypes of AD are caused by heterogeneous genetic and probably environmental factors. Indeed, several genes have been identified that together appear to cause most familial forms of the disease, whereas the ε4 allele of ApoE has been shown to be a significant risk factor for late onset forms of AD (Lamb et al. 1997, 1999, Neve & Robakis 1998, Jakobovits et al. 2000, Kulnane & Lamb 2001, Hock & Lamb 2001, Aliev 2002, Kulnane et al. 2002, Aliev et al. 2002a, de la Torre 2002a,b). Impairment of spatial memory in mice that overexpressing wild type AβPP751 or wild type AβPP 695 and the neuropathology in mice expressing Aβ1-42 have been reported . Expression of AβPP in FAD mutant mice results in deposition of Aβ, while mice expressing the carboxyl terminus 100 or 104 (C100 or C104) amino acids of AβPP demonstrate both neurodegeneration and specific impairment of spatial memory (Duff 1998). Calhoun and coworkers (Calhoun et al. 1998) have shown that the formation of amyloid plaques can lead to region-specific loss of neurons in a transgenic mouse. In addition, it has been shown that mice overexpressing the human mutant amyloid precursor protein (hAPP) show learning deficits, but the apparent lack of a relationship between these deficits and the progressive Aß plaque formation that the hAPP mice display is puzzling (Chen et al. 2000, Refolo et al. 2000). Using a new water-maze training protocol, that PDAPP mice also exhibit a separate age-related deficit in learning a series of spatial locations (Chen et al. 2000). This impairment correlates with Aß plaque burden and is shown in both cross-sectional and longitudinal experimental designs. These findings indicate that Aß overexpression and/or Aß plaques are associated with disturbed cognitive function and, importantly, suggest that some but not all forms of learning and memory are suitable behavioural assays of the progressive cognitive deficits associated with AD-type pathologies (Chen et al. 2000). Later studies have demonstrated that AßPP expression also occurs in different pathological conditions such as after the global ischemia, even without the presence of a genetic abnormality (Lin et al. 2001) indicating central and crucial role of the chronic injury stimuli (e.g. ischemia, hypoxia, virus, toxins etc.) in the pathogenesis of AD. Cerebral amyloid (CA), thought to be produced in the lysosomes of EC (Neve & Robakis 1998), was first proposed as the cause of BBB breakdown, allowing neurotoxic serum proteins access to neuronal cells and beginning the cascade of neurodegeneration. We demonstrated that the C57B6/SJL transgenic mouse model, which overexpressed AβPP (Hsiao et al. 1996) with FAD mutation contains Aβ deposition patterns similar to those seen in cases of AD. In addition, the C57B6/SJL transgenic mouse possesses a beta fibroblast growth factor (bFGF) binding pattern similar to that seen in AD. When tissues from these mice were put through immunohistological assays, the cores of amyloid plaques showed intense staining for the antibody against Aβ (4G8). Additionally, bFGF binding was greatly diminished by heparinase pre-treatment (Shi et al. 1999, 2002). We have reported that mitochondrial abnormalities such as electron dense (ED) mitochondria, mitochondria derived lysosomes and lipofuscin appear to be features of damaged neurons in aged C57B6/SJL Tg (+) mice (Aliev et al. 2000a, Shi et al. 2002a). This indicates that the vascular abnormalities were associated with the selective damage of cortical neurons, raising questions about the relationships between vascular abnormalities, BBB breakdown, neuronal loss and amyloid deposition during the maturation of AD-like pathology in this transgenic mice (Aliev et al. 2000a, 2002). However, no serum amyloid protein (SAP) immunoreactivity was found in the transgenic mouse brain (Shi et al. 1999). Since only peripheral organs synthesize SAP, its presence in the AD brain suggests impairment of the BBB (Price et al. 1997). These results suggest that the pathogenesis of BBB impairment in this mouse model differ from that in AD (Shi et al. 1999).

Recently, YAC transgenic mice that overexpress Aβ have been developed (Lamb et al. 1997, 1999, Jakobovits et al. 2000, Kulnane & Lamb 2001, Hock & Lamb 2001, Kulnane et al. 2002). The YACs

contain the entire ~400kbp human gene encoding AβPP. The gene harbours either (1) the asparagine for lysine and leucine for methionine FAD substitution at codons 670 and 671 ($APP_{K670N/M671L}$), (2) the isoleucine for valine FAD substitution at codon 717 (APP_{V7171}), or (3) a combination of both substitutions (Lamb et al. 1997, 1999, Jakobovits et al. 2000, Kulnane & Lamb 2001, Hock &Lamb 2001, Kulnane et al. 2002). Lowered levels of α-secretase-generated soluble AβPP derivatives are observed in these mice (Lamb et al. 1999, Jakobovits et al. 2000, Kulnane & Lamb 2001, Hock &Lamb 2001, Kulnane et al. 2002). Moreover, there are elevated levels of longer Aβ peptides (species terminating at amino acids 42/43) in YAC transgenic mice that express human $AβPP_{V7171}$, suggesting that these mice should be appropriate for detailed analysis on the *in vivo* effects of AβPP metabolism and Aβ production. Therefore, since YAC transgenic approaches avoid the problems regarding regional and temporal specificity of molecular pathogenesis, they may provide unique insights into the mechanism (s) behind the progression of AD in humans (Aliev et al. 2002). The changes of the vascular wall in YAC AβPP mice, but not age-matched controls, revealed a different degree of amyloid deposition present in vascular wall cells (Aliev et al. 2002). These vessels also showed immunopositive staining for amyloid precursor protein (APP) and were characterized by the presence of a large number of lipid-laden vacuoles in the matrix of endothelial and perivascular cells (Aliev et al. 2002, Shi et al. 2002a). Very often the clusters of APP containing immunopositive gold particles were observed in the neuronal cell bodies of parietal cortical neurons from aged YAC AβPP mice. The ultrastructural abnormalities in vascular wall cells and neurons were very often accompanied by the presence of Aß deposits around the microvessels (Aliev et al. 2002, Shi et al. 2002). This data indicates that disruption of BBB function in vascular EC may be a major factor in lipid accumulation and amyloid deposition during the development of AD-like pathology in YAC AβPP mice (Aliev et al. 2002). This indicates that damage of the vascular endothelium induced by chronic hypoperfusion acts as a primary key factor for oxidative stress and contributes for future neuronal lesions and non-reversible changes in neurons that induce AβPP overexpression and Aβ depositions, permanent features of AD (Aliev et al. 2000a, 2002, Shi et al. 2002a). These findings raise questions regarding the direct relationship between vascular abnormalities, BBB breakdown, neuronal loss, mitochondrial lesions and Aβ deposition during the maturation of AD-like pathology (Aliev et al. 2000a, 2002, Shi et al. 2002a). Therefore, cellular and subcellular investigations into both the mechanisms behind Aβ deposition development and the possible accelerating effects of environmental factors such as chronic hypoxia/reperfusion may open the door to new pharmacological treatments of AD. We hypothesize that an imbalance between NOSs and ET-1, along with antioxidant system deficiency, is predominant in the brains of stroke and AD patients. Elevated chronic hypoperfusion and physical distortion of the tissue are likely to contribute to the collapse of post-ischemic/hypoxic or AD vessels. The sustained hypoperfusion and oxidative stress of brain tissues could also stimulate the expression of NOSs and ET-1 in brain cells and probably increases the accumulation of oxidative stress products, therefore contributing to BBB breakdown and brain parenchymal cell damage.

Subcellular Mechanisms for the Development of Human AD

Ultrastructural features of the brain microvessels EC and perivascular cell from aged matched control cases brain did not show visible changes in their ultrastructure. Mitochondria in the EC were intact (figure 1). Conversely, observations of cortical microvessels from AD brain biopsies were characterized by the differential degree of damage (figure 1). However lesions were heterogeneous (figure 1). In some areas microvessels showed a high degree of lesions such as the presence of a cluster of mitochondria derived lysosomes and necrotic changes in the ultrastructure of the vascular EC and perivascular cells (figure 1). Very often capillary endothelium shows the presence of *"giant"* sized lipid vacuoles in their matrix. Transformation of the mitochondria to the mitochondria derived lysosomes is generalized to all brain cellular compartments (figure 1). In addition, EC occupied only a small part of the vessel

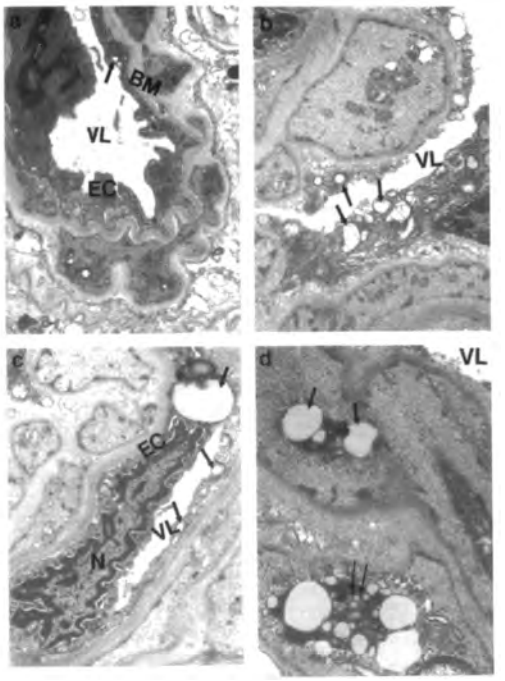

Figure 1 Ultrastructural features of the brain microvessels from the AD brain biopsy. a- Endothelial cell (EC) and perivascular cell from non-damaged vessels show absence pathological change in their ultrastructure. Mitochondria in EC are intact. Original magnification: X 8,300. b- The microvessels with severely damage such as presence of a clusters of mitochondria derived lysosomes (single arrow) and necrotic changes in the ultrastructure of the perivascular cells. Original magnification: X 10, 000. c- Capillary endothelium shows the presence of a giant sized lipid vacuoles in their matrix. Mostly mitochondria already transformed to the mitochondria derived lysosomes (single arrow). Original magnification: X 10,000. d- EC occupied only the small part of the vessel wall. Perivascular cells show the presence of large sized mitochondria derived vacuoles (single arrow) in their matrix. Some of vacuoles were co-exists with the presence of lipid laden vacuolar degenerative structure (indicate by double arrow). Original magnification: X 10, 000. Abbreviations used in figures: BM-basal membrane; EC- endothelial cell; N-Cell Nucleus; VL-Vessel lumen.

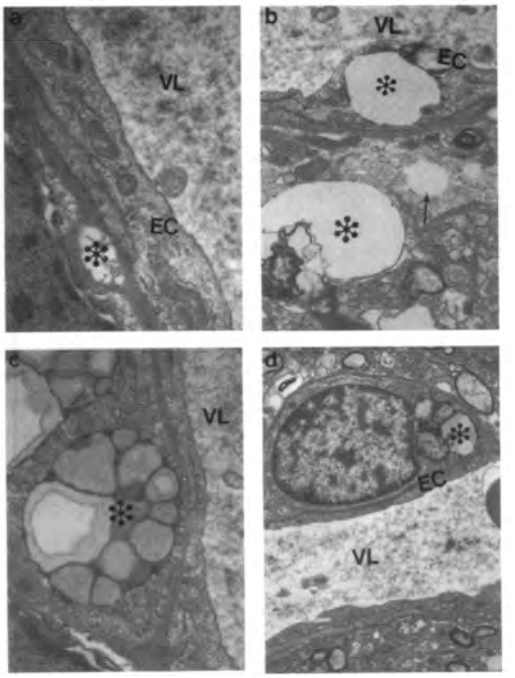

Figure 2 The ultrastructural characteristics of the brain biopsy from AD patients. a- Non-damaged microvessels endothelium show intact morphology. However, the luminal plasmalemma of this EC was sharply protruded to the vessel lumen, and the perivascular spaces contain vacuolar structure (asterisk). Original magnification X 20,000. b- Vascular EC and perivascular cell shows the presence of giant sized vacuolar degenerative structure in their matrix (asterisk) and this is co-exists with the formation of the mitochondria derived lysosomal structure (arrow). Original magnification X10, 000. c- Perivascular cells contain giant sized lipid laden vacuolar degenerative structure with amyloid deposition (asterisk). Original Magnification X8, 300. d- Microvessels with early stage of the damage. Perivascular astrocytes shows the lipid laden vacuolar structure (asterisk) with residues of the cytoplasmic organelles (e.g. membranous structure). Original Magnification X 10,000. Abbreviations used in figures: EC- endothelial cell; VL-Vessel lumen.

wall. Perivascular cells show the presence of large number of the mitochondria derived vacuoles in their matrix (figure 1). Sometimes microvessels endothelium, at the early stages of AD, did not show any damage in their ultrastructure. However, the luminal plasmalemma of this EC sharply protruded into the vessel lumen, indicating the effect of hypoperfusion before any visible ultrastructural damage. Cellular organelles including mitochondria still intact. Perivascular spaces contain some vacuolar degenerative structure (figure 2a). AD affected regions very often characterized the presence of vascular endothelium and perivascular cell contained giant-sized vacuolar degenerative structures in their matrix. These abnormalities coexist with the formation of mitochondria derived lysosomal structures (figure 2 b). Perivascular cells from lesioned vessels show the presence of a giant-sized lipid-laden vacuolar degenerative structure with amyloid deposition in the cytoplasmic matrix (figure 2c). Very often, the cytoplasmic matrix contained only residues of the cytoplasmic organelles (e.g. membranous structure) (figure 2d).

Recently we have demonstrated that the ultrastructural features of the cortical neurons from

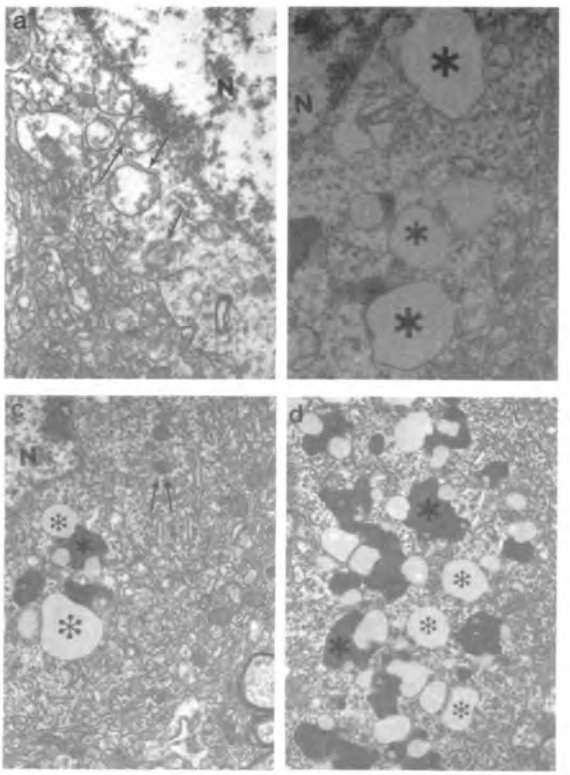

Figure 3 The ultrastructural characteristics of the neuronal mitochondria damage from AD brain biopsy. Neurons with different degree of ultrastructural lesions. In the neuronal cell body partially and completely damaged mitochondria (indicated by single arrows and asterisk respectively) were co-exists with lipofuscin formation and mitochondria appeared to be a major substrate for their formation. Large number of electron dense hypoxic mitochondria were seen throughout cell body and characterized the abnormal mitochondrial cristae (indicates by double arrow in figure c). Original magnification: a and b X 16,000 respectively. c and d X 10,000 respectively.

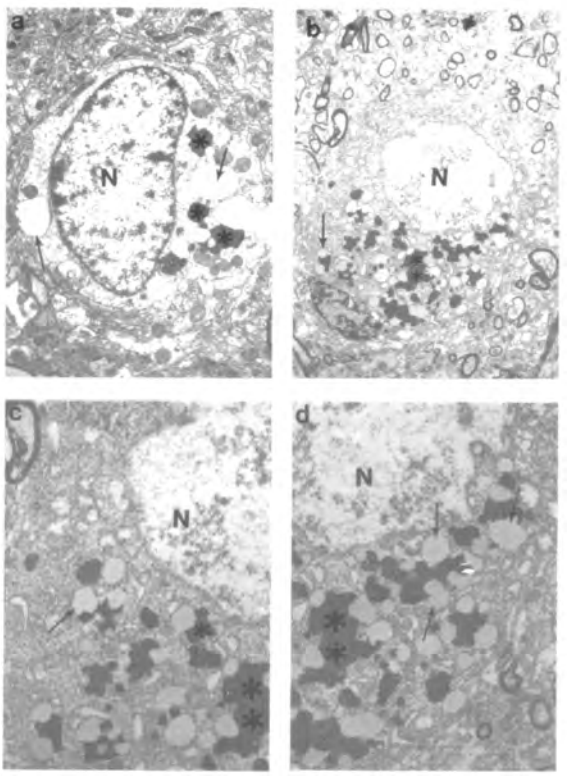

Figure 4 Subcellular features of the non-reversible damaged neurons from AD brain biopsy. a- Neuronal cell body shows almost absence any kind of intact cellular organelles. Different stages of mitochondria lesions such as formation of mitochondria derived lysosomes (indicates by single arrow) and lipofuscin (asterisks) appeared to be their permanent features. Original magnification X 6,600. b-d- Mitochondria derived lysosomes and lipofuscin (double asterisk) are the permanent features of the neuronal abnormality of the cortical neurons. b-The cortical neuron under the lower magnification (X3, 300). Left bottom and central bottom portions of this neuron under the higher magnification (X6, 600 respectively c and d). Abbreviations used in figures: N-Cell Nucleus.

AD brain biopsy are characterized selective localization of mitochondria abnormality in the cell body (Aliev et al. 1999, 2000a, 2002, Hirai et al. 2001, Shi et al. 2002a). The majority of the neurons, which closely associated with the lesioned vessels, possessed a different degree of ultrastructural abnormality (figure 3). In the neuronal cell body, the presence of partially and completely damaged mitochondria, were associated with lipofuscin formation and mitochondria appeared to be a major substrate for this process (figure 3c-d). A large number of electron dense hypoxic mitochondria were seen throughout the cell body and characterized the abnormal mitochondrial cristae (figure 3 c-d). In many cases, neuronal cell body showed an absence of cellular organelles (figure 4a).

Different stages of mitochondrial abnormality, such as formation of mitochondria derived lysosomes and lipofuscin, were seen in damaged but not in normal neurons. The mitochondria-derived lysosomes and varied dense and sized lipofuscin deposits were the permanent features of the neuronal abnormality (figure 4b-d). Mitochondria lesions and lipofuscinogenesis were also generalized to the other cellular compartments of the brain parenchyma. Very often, glial cells at the damaged area, also characterized by the accumulation of lipofuscin and mitochondria-derived lysosomes appeared to be a major component and source for these substrates (figure 5a-b). In addition, glial cells also show the intracellular accumulation of different sized

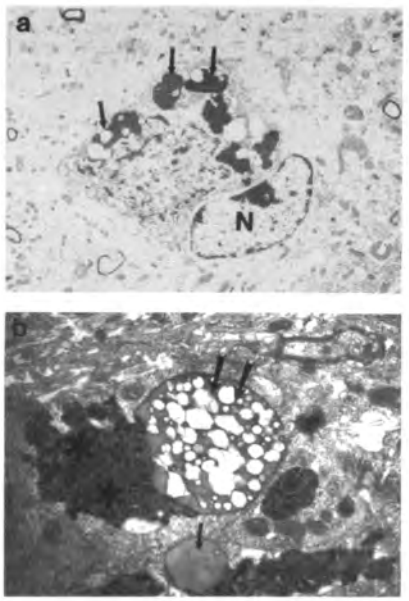

Figure 5 The characteristics of the glial cell damage from AD brain biopsy. a-Glial cells characterized with the accumulation of lipofuscin and mitochondria derived lysosomes appeared to be major component and resources for this substrate. Original magnification X 5,000. b- Glial cells show the intracellular accumulation of the different sized amyloid deposits. Giant lipid-laden vacuole (double arrow) and mitochondria derived lysosomes (arrow) associated with amyloid deposits. Original magnification X 14,000. Abbreviations used in figures: N-Cell Nucleus.

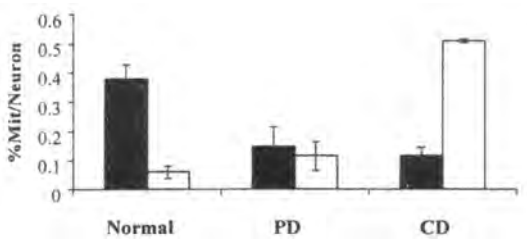

Figure 6 The percentage of different types of mitochondria with cristae (normal) and with partial (PD) or complete (CD) cristae destruction in brain biopsy samples from control (n = 6) and AD (n = 8) patients.

amyloid deposits, and they are accompanied by the presence of giant-sized lipid-laden vacuoles and mitochondria derived lysosomes (figure 5b).

Quantitative morphometric measurements of the percentage of the different types of mitochondria (normal, partially damaged and completely damaged) indicate that aged-matched control groups show a significantly higher percentage of normal mitochondria compared to AD cases (figure 6). However the percentage of the completely damaged mitochondria was significantly lower in control groups compared to AD (figure 6). Whereas no significant differences between partially damaged mitochondria were seen in both groups, indicating that aging it induces damage to mitochondria. However, the main differences between the aged-matched controls and AD cases appeared to be significant differences in the percentage of the normal and completely damaged mitochondria (figure 6).

Cytological *in situ* hybridization studies using human wild-type and 5kB deleted mtDNA probes have found that detection of mtDNA signals were associated with severely damaged or mitochondria-derived lysosomal structures (figure 7a-c). However, the area containing lipofuscin did not show any mtDNA containing positive signals (figures 7a and c). Very often, clusters of 5kb deleted mtDNA containing gold particles (17 nm) were mostly localized in mitochondria-derived lysosomal structures (figure 7c), indicating that mtDNA deletions and turnover occur at the late stages of the mitochondrial lesion formation. Similarly, wild type mtDNA probes lipofuscin containing areas of the neuronal cell body were free from any 5kb deleted mtDNA positive signals (see figure 7c). In contrast to this observation, aged-matched control case hippocampal neuronal mitochondria did not show mtDNA positive immunoreactivity nor contain gold particles in their matrices. Only infrequently, a few wild type mtDNA positive signals containing gold particles were seen (figure 7d).

Our extended detailed ultrastructural analysis demonstrates that the mitochondrial abnormality in neurons are associated with increased markers of lipid peroxidation, detected by pre-embedding immunocytochemistry methods using antibody against to the free lipoic acid. Free lipoic acid-containing colloidal gold particles were seen in the membrane of the partially and/or completely damaged mitochondria (figure 8a). The external membrane of the damaged, but not normal mitochondria, very often showed clusters of free lipoic acid-containing immunopositive gold particles (figure 8a). However, the matrix of lipofuscin or amyloid like structure was free from lipoic acid-containing gold particles (17 nm). In addition, the presence of clusters, of free lipoic acid-containing gold particles were associated with the membrane of the granular vacuolar degenerative

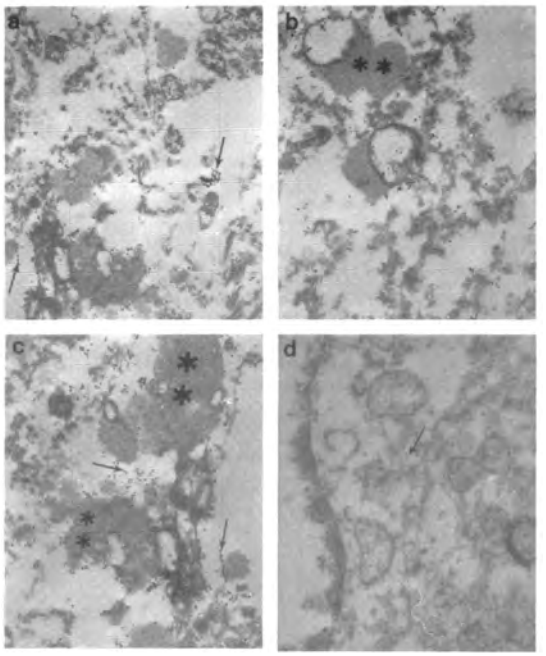

Figure 7 The features of wild (a, c and d) and 5 kb deleted (b) mitochondria DNA (mtDNA) detection in the hippocampus of the postmortem human AD (a-c) and aged matched control case (d). a and c- Postmortem AD hippocampus shows that wild type mtDNA containing signals (17 nm colloidal gold) detection were associated with severely damaged mitochondria and mitochondria derived lysosomes (single arrows). Areas containing lipofuscin (double asterisk) did not show any mtDNA containing positive signals. Magnification X 26,000 and X 30,000 respectively a and c. b- Postmortem AD brain. 5kb deleted mtDNA containing gold particles (17 nm) were mostly located in mitochondria derived lysosomes (dark dots). Lipofuscin containing areas of the neuronal cell body were free from any 5kb deleted mtDNA positive signals. Original magnification X 25,000. d- Aged-matched control case hippocampal neuron. Mitochondria did not show any mtDNA positive containing gold particles in their matrix. Only occasionally few wild type mtDNA containing signals were seen (colloidal gold, 17 nm). Original magnification X 30,000.

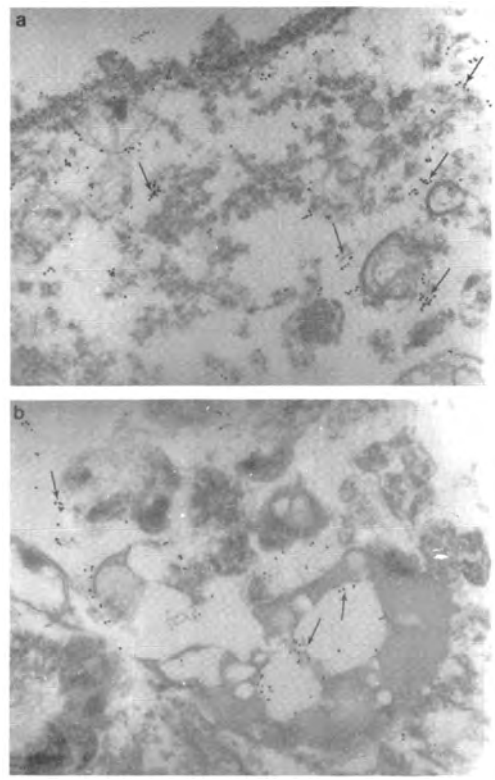

Figure 8 The immunochemical detection of the lipid peroxidation markers on the hippocampal neurons of the postmortem AD brain visualized by immunostaining using antibody against free lipoic acid. a-Free lipoic acid containing colloidal gold particles were seen in the membrane of the partially and/or completely damaged mitochondria. The external membrane of the damaged but not normal mitochondria shows cluster of the lipoic acid containing immunopositive gold particles (indicated by single arrow). Original Magnification X 26,000. b- The hippocampal neurons from AD patients. Free lipoic acid positive gold particles was absent in the matrix of lipofuscin or central core of the granulovacuolar degenerative structure. The clusters of free lipoic acid containing gold particles always associated with the membrane of the granular vacuolar degenerative structure (indicated by single arrows). Original magnification X 26,000.

structure (figure 8b). Moreover, increases in the immunoreactivity of lipid peroxidation markers are associated with the RNA oxidation (staining by 8-OHG). The clusters of 8-OHG containing immunopositive gold particles (17 nm) were localized in the matrix of completely and/or partially damaged mitochondria (figure 9a). Large numbers of immunopositive gold particles were also associated with cytoplasmic matrix itself. The same was true for free lipoic acid immunostaining, 8-OHG containing gold particles were absent in the matrix of lipofuscin (figure 9b). In addition, the cytoplasmic matrixes, especially of the regions were damaged mitochondria or mitochondria-lipofuscin present, showed clusters of 8-OHG containing immunopositive gold particles (figure 9a-b). Our quantitative study clearly indicates that the extent of oxidative damage (e.g. 8OHG staining) is highly dependent on the degree of mitochondrial abnormalities (figure 10).

We have found that highly overexpressed immunoreactivity for lipid peroxidation, RNA oxidation and mitochondria damages are associated with an increase in the metal iron activity at the same cellular compartment especially in the neurons with non-reversible damage. We obtained evidence for alterations of iron metabolism in AD, including increased levels of free iron as well as altered levels

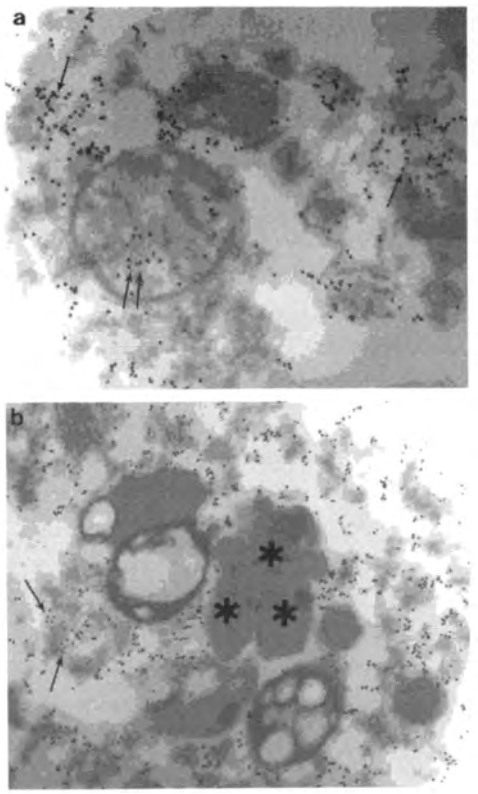

Figure 9 Features of the RNA oxidative markers stained by 8-OHG in vulnerable neurons from postmortem AD brain hippocampus. a-The clusters of 8-OHG containing immunopositive gold particles (17 nm) were localized in the matrix of completely (single arrow) and/or partially damaged (double arrow) mitochondria. Large numbers of immunopositive gold were also associated with cytoplasmic matrix (free ribosomal structure) of the vulnerable neuron. Original magnification X 33,000. b- 8-OHG containing gold particles were absent in the matrix of lipofuscin (asterisk). The immunopositive gold particles were localized throughout cytoplasmic matrix, especially with severely damaged mitochondria. Original magnification X 33,000.

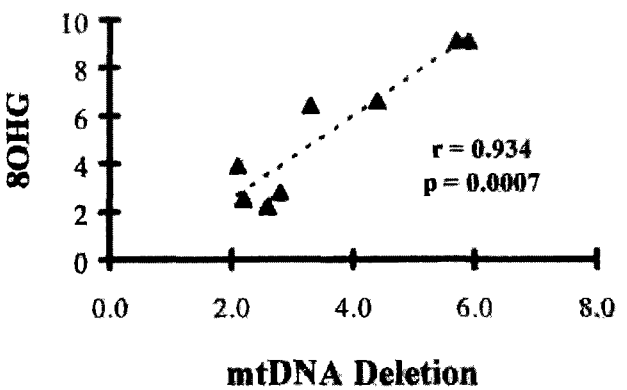

Figure 10 The extent of oxidative damage (8OHG) is highly dependent on the degree of mitochondrial abnormalities, in arbitrary units.

of iron transport and storage proteins. Furthermore, using the H_2O_2-dependent oxidation of 3, 3'-diaminobenzidine (DAB) to determine sites of non-enzymatic catalytic redox activity in tissue sections from AD and control cases we directly demonstrated that at least some of this iron associated with AD pathology is redox active (figure 11a-b). DAB oxidation was inhibited by chelation of metals with DTPA or DFX, with the former being more effective on an equimolar basis for NFT, senile plaques SP, and the cytoplasmic vesicles (see figure 11a). More specifically, prior treatment of the sections with 100 mM or 10 mM DTPA abolished all or nearly all, respectively, lesion-associated oxidation of DAB, and 1 mM DTPA still inhibited more than half of the DAB staining (data not shown). In contrast, 100 mM deferoxamine (DFX) was incompletely effective in inhibiting the lesion- and vesicle-associated DAB oxidation, 10 mM DFX reduced the DAB staining by only about half, and the inhibitory effect of 1 mM DFX was barely noticeable (data not shown). Following chelation of metals with 100 mM DFX, lesion-associated H_2O_2-dependent DAB oxidation could be re-established by incubation of the tissue sections either with a mixture of 0.01mM $FeCl_2$ and 0.01mM ferric citrate or with 0.01mM $CuSO_4$, with copper being more effective than iron. These results indicate that NFT, SP, and vesicles bind endogenous redox active transition metals in a manner that permits them to catalyze H_2O_2 oxidation of DAB at the site of metal binding, implicating cycling of the metal ions between oxidized and reduced states. There are only limited types of protein sites for adventitiously-bound metal ions expected to have sufficient affinity for both reduced and oxidized states thereof as to resist complete removal of the metals by chelators. Our focus has been on iron and copper because these are the most common redox-active circulating metals and because the criteria for redox activity of other potential transition metals (cobalt, nickel, manganese, and chromium) are more stringent and/or typically limited to specially designed enzyme active sites.

Although *in situ* histochemical techniques lack the sensitivity to detect copper, we are aware that copper (as well as iron) could contribute to the redox activity. Therefore, we used the relative

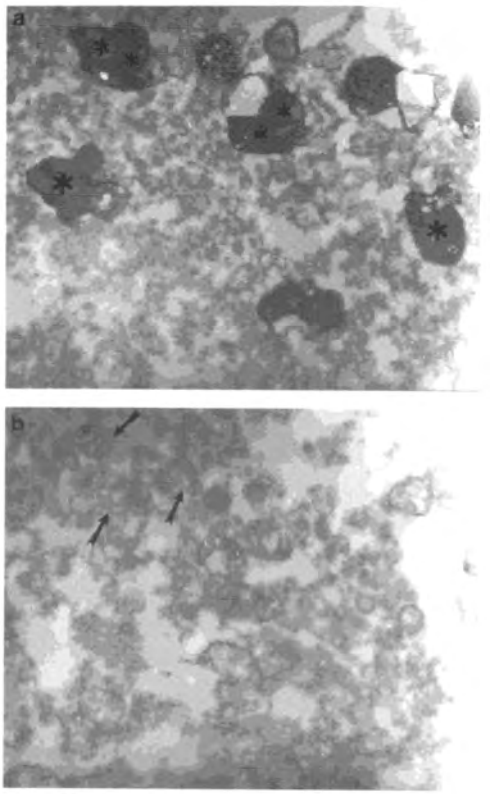

Figure 11 Ultrastructural features of the metal redox activity in human AD and aged matched control brain. a- Vulnerable neuron from postmortem AD case shows high redox activity localized in the population of electron dense (ED) *"hypoxic"* and/or mitochondria derived lipofuscin (indicated respectively by single and double asterisk). High intensity of the DAB reaction was also associated with the vesicular structure of the neuronal cell body. Original magnification X13, 000. b- Aged-matched control case hippocampal neuron shows very low of DAB positive staining. Low activity was also seen in mitochondria (indicated by single arrow). Original magnification X 16,000.

effectiveness of copper- and iron-selective chelating agents to remove the lesion-dependent redox activity, to provide evidence for both copper- and iron-mediated redox activities in AD at the electron microscopic level (figure 11). Ultrastructural features of the metal redox activity in human postmortem AD and age-matched control brain samples, visualized by staining diaminobenzidine hydrochloride (DAB) without osmification and uranylacetate and lead citrate co-staining, indicate that only vulnerable neurons from AD cases show high redox activity localized in the population of hypoxic electron dense and or mitochondria derived lipofuscin. High intensity of the DAB reaction was also associated with the cytoplasmic vesicles of the neuron. Contrary to these observations, aged-matched control case hippocampal neurons did show very low DAB positive staining in cellular and subcellular compartment including the mitochondria (figure 11b).

Based on the literature and on evidence presented in this review we theorize the hypothetical time line of the damage in neuronal structure and relationships to lipid peroxidation, nitration and oxidative stress during the maturation of AD much more complex than that proposed hypothetical schematic drawing (figure 12). With the onset of AD, normal neurons develop numerous forms of oxidative damage including nitration (nitrotyrosine), lipid peroxide adducts (lipid peroxidation), and nucleic acid oxidation prior to the formation of pre-neurofibrillary tangles (pre-NFT) that induces non-reversible damage of the neurons and therefore finally failure of neurotransmission that occur in AD brain.

Similarity and Differences between the Subcellular Mechanisms of the Development of Human AD and AD-like Pathology in a Transgenic Mouse

Recently we applied the **C57B6/SJL** transgenic mouse model overexpressing Ab to assess the binding of bFGF and SAP to Ab as a measure of BBB integrity. Adjacent sections of brain were stained with 4G8, a monoclonal antibody to amyloid, with bFGF binding followed by 48.1, a monoclonal antibody against bFGF. The binding of bFGF in this model is similar to that of AD cases in which bFGF binds specifically to Ab neuritic plaques and the BM

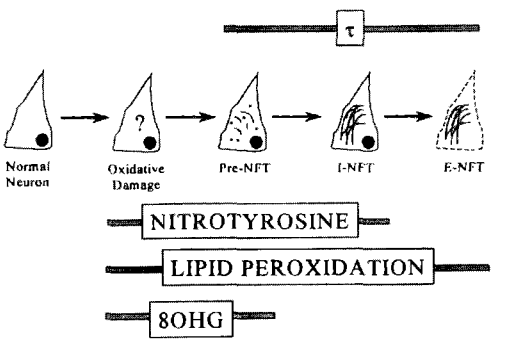

Figure 12 Hypothetical time line of the damage in neuronal structure in AD. With the onset of AD, normal neurons develop numerous forms of oxidative damage including nitration (nitrotyrosine), lipid peroxide adducts (lipid peroxidation), and nucleic acid oxidation prior to the formation of neurofibrillary tangles (pre-NFT).

of cerebral microvessels (Shi et al. 1999). In addition, the cores of amyloid plaques are intensely stained with the 4G8, and bFGF binding is colocalized with amyloid immunoreactivity as visualized by polyclonal antiserum to amyloid. Our ultrastructural study indicates that bFGF immunostaining in aged **C57B6/SJL** mice appeared to be that of immunopositive peroxidase-antiperoxidase (PAP) products or gold particles that were associated with damaged, but not normal neurons (Aliev et al. 2000a, 2002). Moreover, this is associated with the different degree of Aβ immunostaining in the neuronal cell body and vascular wall cells (Aliev et al. 2002). In addition, we have found the degree of mitochondrial abnormality, such as electron dense (ED); or mitochondria derived lysosomes and lipofuscin appeared to be features of damaged neurons in aged (24 m old) **C57B6/SJL** TG (+) but not age-matched control mice, indicating that the vascular abnormality was associated with the selective damage of cortical neurons, and raising questions that the direct relationships between vascular abnormality, BBB breakdown, neuronal loss and amyloid depositions during the maturation of AD like pathology (Aliev et al. 2002).

The ultrastructural features of vascular lesions and mitochondria changes in neuronal cell bodies in transgenic and non-transgenic age-matched control mice were analyzed following perfusion fixation (Aliev et al. 2000a). EM immunocytochemistry using monoclonal antibody reveals different sizes of fibrils and extracellular types of amyloid deposits of brain tissues in YAC AßPP transgenic mice (Aliev et al. 2000a, 2002). In addition, the amyloid depositions were associated with the formation of parietal helical filamental (PHF) structures, which is a permanent feature of neuronal lesions in AD brains (Aliev et al. 2000a).

The changes of the vascular wall in YAC AßPP Tg (+), but not age-matched control mice appeared to be the presence of different degree of amyloid depositions in vascular wall cells (Aliev et al. 2002). These vessels were also showed the immunopositive staining for APP and characterized the presence of a large sized of lipid-laden vacuoles in the matrix of EC and perivascular cells (Aliev et al. 2002). Moreover, these changes were also generalized to cortical microvessels as it to AD (Aliev et al. 2002). The ultrastructural abnormalities of vascular wall cells were dependent on the presence of Aß deposits around the microvessels (Aliev et al. 2002).

In contrast with these observations age-matched control mice brain vessels did not show any particular changes in the ultrastructure of vascular EC at different levels of microcirculation. Only sometimes did a very small amount of lipid droplets appear in the matrix of perivascular cells (Aliev et al. 2002). This data clearly indicates that disruption of BBB function in vascular EC may be major factor in lipid accumulation and amyloid deposition during the development of AD-like pathology in YAC AßPP Tg (+) mice without cholesterol feeding (Aliev et al. 2002; Shi et al. 2002a). Moreover, cortical neuronal cell body in YAC AßPP mice were characterized by different degree of ultrastructural alterations in their mitochondrial structures as same as to AD (Aliev et al. 1999, 2000a). Particularly giant and ED mitochondria appeared to be permanent features of the neuronal abnormality, and this is associated with the APP overexpression (Aliev et al. 2000a). We have also found that same as to AD brain different stages of mitochondria lesions in the neuronal cell body appeared to be associated with the absence of microtubule and with lipofuscin formation, that as same as observations were seen in the populations of vulnerable neurons in AD brain biopsy indicating that the cytoskeletal abnormality of neurons (Cash et al. 2003). Age-matched control mice did not show any particular changes in their neuronal ultrastructure (Aliev et al. 2000a). *In situ* hybridization analysis with mouse and human mtDNA probes found abundant deleted mtDNA in YAC AßPP compared to age-matched controls (Aliev et al. 2000a, Aliev et al. 2002). Moreover, the majority of mtDNA deletion was found in mitochondria-derived lysosomes in regions closely associated with the lipofuscin, and suggests that proliferation, deletion and duplication of mtDNA occurs in mitochondria, many of which have been fused with lysosomes, in YAC AßPP mice (Aliev et al. 2000a).

These finding suggest that abnormalities in mitochondrial structures and mtDNA are feature of damaged neurons in YAC AßPP mice, and may play

a key role in the pathogenesis of AD (Aliev et al. 2000a). In addition, we found that neurons in samples from AD were dominated by abnormal mitochondria as compared to the control group (Hirai et al. 2001). By *in situ* hybridization analyses, with a chimeric cDNA probe to the 5kb common deletion (Hirai et al. 2001), we found that deleted mtDNA is increased at least 3 fold for the AD cases compared to the controls. In quantitative analysis of the mtDNA deletion and 8OHG in the same cases, we found a strong positive correlation (r = 0.934; figure 10). Ultrastructural localization of mtDNA *in situ* hybridization with colloidal gold showed that deleted mtDNA is mainly found in abnormal mitochondria, i.e., those lacking cristae, swollen and in many cases fused with lipofuscin (Hirai et al. 2001).

These findings suggest that the mtDNA *in situ* hybridization detected mtDNA proliferation, deletion and duplication in abnormal mitochondria, many of which have been fused with lysosomes indicating, they are being turned over. The detailed analysis of 8-OHG immunostaining demonstrated that only vulnerable neurons show immunopositive staining for 8-OHG in AD, but not in age-matched controls (figure 9). By using ultrastructural analysis we have found that 8-OHG immunostaining was selectively associated in vulnerable neurons and microvessels of AD brain (Hirai et al. 2001, Aliev et al. 2002a). The 8-OHG immunogold labelling (17 nm) is seen throughout the cytoplasm, including the damaged mitochondria or electron dense abnormal mitochondria (Hirai et al. 2001, Aliev et al. 2002a). However, we did not find 8-OHG in normal mitochondria or in lipofuscin (see figure 9). The capillary EC and perivascular pericytes show the high intensity of 8OHG immunostaining (Aliev et al. 2002a).

We speculate that the oxidative stress markers seen in the AD brain selectively affect the population of vulnerable neurons, vascular EC and perivascular cells. These observations suggest that hypoperfusion-induced oxidative stress plays a key role in the pathogenesis of vascular and non-vascular cell lesions during the maturation of AD. Moreover, detailed immunocytochemical analyses using colloidal gold probes indicate that the vascular wall in YAC AßPP transgenic mice possesses atherosclerotic lesions, while control and non-damaged vessels from YAC AßPP mice do not show APP immunopositivity (Aliev et al. 2002). Very often the clusters of APP positive immunoreactivity were observed in the neuronal cell bodies of parietal cortical neurons from aged YAC AßPP transgenic mice (Aliev et al. 2000a, 2002). *In situ* hybridization using wild and deleted mtDNA probes (human and mice specific) revealed mtDNA containing gold particles in YAC AßPP, but not in control mouse hippocampus (Aliev et al. 2000a). The main source of the mtDNA probes is in damaged mitochondria and mitochondria derived lysosomes, but not in lipofuscin (figure 7).

We have found that wild and chimeric mtDNA was also detectable in YAC AßPP Tg (+) mouse microvessels but not in control, age-matched brain tissue (Aliev et al. 2002). In addition, vessels with atherosclerotic lesions show that endothelium and perivascular cells contain clusters of wild and deleted mtDNA containing positive signals (Aliev et al. 2002). These observations suggest that the key role of hypoperfusion, mitochondrial abnormality and oxidative stress in the pathogenesis of vascular and non-vascular cells lesions during the development of AD-like pathology in YAC AßPP mice (Aliev et al. 2002) and at the many point overlap with the neuropathology of human AD.

Conclusion and Future Remarks

The evidence that garnered from this review will focus our future direction and provide a clearer understanding between the relationship the number of age-related disorders including atherosclerosis, ischemia/reperfusion, stroke, neurodegenerative diseases, especially AD that very often coexist with cognitive impairments. Further, we indicate that chronic vascular hypoperfusion is a main part of their common underlying mechanisms. Chronic hypoperfusion appears to be a central initiating factor for vascular abnormality, mitochondrial damage and an imbalance in the activity of NOS isoforms, ET-1, oxidative stress markers, mtDNA and mitochondrial enzymes in the vascular wall and in brain parenchymal cells is predominant in CVA and AD. This imbalance augments chronic hypoperfusion and follows oxidative stress. Therefore, determining the mechanisms behind these imbalances may provide crucial information in

the development of new, more effective therapies for stroke and AD patients in the near future.

Future studies must seek to answer the following questions: (1) What are the major factors altering and/or controlling cerebral blood flow during the progression of chronic hypoperfusion and/or the development of atherosclerotic changes in brain microvessels? (2) What are the roles of vasoactive substances (namely NO and ET-1) during the development of these pathophysiology? (3) Does chronic hypoperfusion with concomitant oxidative stress accelerate the vascular and neuronal lesions (including the mtDNA deletions) during normal aging and/or when brain exposed to chronic hypoperfusion induced factors?

Resolving these issues will allow for novel therapeutic approaches that will modify the natural history of these chronic age onset disorders in 21st century.

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References

Acuna-Castroviejo D, Martin M, Macias M, Escames G, Leon J, Khaldy H and Reiter R J 2001 Melatonin, mitochondria, and cellular bioenergetics; *J. Pineal Res.* 30 65-74

Al Abdulla N A and Martin L J 1998 Apoptosis of retrogradely degenerating neurons occurs in association with the accumulation of perikaryal mitochondria and oxidative damage to the nucleus; *Am. J. Pathol.* 153 447-456

Aliev G 2002 Is non-genetic Alzheimer's disease a vascular disorder with neurodegenerative consequences? *J. Alzheimer's Disease* 4 513-516

_____ and Burnstock G 1998 Watanabe rabbits with heritable hypercholesterolaemia: a model of atherosclerosis; *Histol. Histopathol.* 13 797-817

_____, Bodin P and Burnstock G 1998 Free radical generators cause changes in endothelial and inducible nitric oxide synthases and endothelin-1 immunoreactivity in endothelial cells from hyperlipidemic rabbits; *Mol. Genet. Metab.* 63 191-197

_____, Cirillo R, Salvatico E, Paro M and Prosdocimi M 1993a Changes in vessel ultrastructure during ischemia and reperfusion of rabbit hindlimb: implications for therapeutic intervention; *Microvasc. Res.* 46 65-76

_____, Miah S, Turmaine M and Burnstock G 1995 An ultrastructural and immunocytochemical study of thoracic aortic endothelium in aged Sprague-Dawley rats; *J. Submicrosc. Cytol. Pathol.* 27 477-490

_____, Mironov A, Cirillo R, Mironov A J, Gorelova E and Prosdocimi M 1993b Evidence for the presence of early vascular lesions in newborn Watanabe heritable hyperlipidemic (WHHL) rabbits; *Atherosclerosis* 101 17-24

_____, Ralevic V and Burnstock G 1996 Depression of endothelial nitric oxide synthase but increased expression of endothelin-1 immunoreactivity in rat thoracic aortic endothelium associated with long-term, but not short-term, sympathectomy; *Circ. Res.* 79 317-323

_____, Shi J, Perry G, Lamb B T, Lamanna J C and Friedland R P 2000a Neuronal mitochondrial abnormalities in yeast artificial chromosome (YAC) transgenic mice overexpressing amyloid precursor protein (APP); *Abstract in: Society for Neuroscience* 30 (26) 301.4

_____, Shi J, Perry G, Friedland R P and Lamanna J C 2000b Decreased constitutive nitric oxide synthase, but increased inducible nitric oxide synthase and endothelin-1 immunoreactivity in aortic endothelial cells of donryu rats on a cholesterol-enriched diet; *Anat. Rec.* 260 16-25

_____, Smith M A, Seyidova D, Neal M L, Lamb B T, Nunomura A, Gasimov E K, Vinters H V, Perry G, Lamanna J C and Friedland R P 2002a The role of oxidative stress in the pathophysiology of cerebrovascular lesions in Alzheimer's disease; *Brain Pathol.* 12 21-35

_____, Smith M A, Seyidova D, Neal M L, Shi J, Loizidou M, Turmaine M, Friedland R P, Taylor I, Burnstock G, Perry G and Lamanna J C 2002b Increased expression of NOS and ET-1 immunoreactivity in human colorectal metastatic liver tumours is associated with selective depression of constitutive NOS immunoreactivity in vessel endothelium; *J. Submicrosc. Cytol. Pathol.* 34 37-50

_____, _____, Turmaine M, Neal M L, Zimina T V, Friedland R P, Perry G, LaManna J C and Burnstock G 2001a Atherosclerotic lesions are associated with increased immunoreactivity for inducible nitric oxide synthase and endothelin-1 in thoracic aortic intimal cells of hyperlipidemic Watanabe rabbits; *Exp. Mol. Pathol.* 71 40-54

_____, Smith M A, Vinters H, Johnson A B, Nunomura A and Perry G 1999 Mitochondria abnormalities mark vulnerable neurons in Alzheimer's disease; *J. Neuropathol. Exp. Neurol.* 58 511

Aliev G, Perry G, Lamb B T, Seyidova D, Neal M L, Siedlak S L, LaManna J C, Smith M A and Friedland R P 2001b Aged YAC AbPP transgenic mice develop atherosclerotic lesions and mitochondria DNA deletions in brain microvessels without exogenous cholesterol supplementation; *Abstracts In: Society for Neuroscience* 752.12

_____, Seyidova D, Lamb B T, Neal M L, Siedlak S L, Vinters H, LaManna J C, Friedland R P, Head E, Perry G and Cotman C W 2002 Atherosclerotic lesions and mitochondria DNA deletions in brain microvessels as a central target for the development of human AD and AD-like pathology in a aged YAC AßPP transgenic mice; *Ann. N.Y. Acad. Sci.* 977 45-64

Almeida A, Bolanos J P and Medina J M 1999 Nitric oxide mediates glutamate-induced mitochondrial depolarization in rat cortical neurons; *Brain Res.* 816 580-586

Bates T E, Loesch A, Burnstock G and Clark J B 1995 Immunocytochemical evidence for a mitochondrially located nitric oxide synthase in brain and liver; *Biochem. Biophys. Res. Commun.* 213 896-900

_____, _____, _____and _____1996 Mitochondrial nitric oxide synthase: a ubiquitous regulator of oxidative phosphorylation? *Biochem. Biophys. Res. Commun.* 218 40-44

Beal M F 1995 Aging, energy, and oxidative stress in neurodegenerative diseases; *Ann. Neurol.* 38 357-366

Beckman J S 1991 The double-edged role of nitric oxide in brain function and superoxide-mediated injury; *J. Dev. Physiol.* 15 53-59

_____, Beckman T W, Chen J, Marshall P A and Freeman B A 1990 Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide; *Proc. Natl. Acad. Sci. U.S.A.* 87 1620-1624

Beckman J S, Carson M, Smith C D and Koppenol W H 1993 ALS, SOD and peroxynitrite; *Nature* 364 584

Beckman J S, Chen J, Ischiropoulos H and Crow J P 1994 Oxidative chemistry of peroxynitrite; *Methods Enzymol.* 233 229-240

Beckman K B and Ames B N 1998a Mitochondrial aging: open questions; *Ann. N. Y. Acad. Sci.* 854 118-127

_____and _____1998b The free radical theory of aging matures; *Physiol. Rev.* 78 547-581

_____ and _____1999 Endogenous oxidative damage of mtDNA; *Mutat. Res.* 424 51-58

Bednar M M, Raymond S, McAuliffe T, Lodge P A and Gross C E 1991 The role of neutrophils and platelets in a rabbit model of thromboembolic stroke; *Stroke* 22 44-50

Bogumil R, Knipp M, Fundel SM and Vasak M 1998 Characterization of dimethylargininase from bovine brain: evidence for a zinc binding site; *Biochemistry* 37 4791-4798

Bonilla E, Tanji K, Hirano M, Vu T H, DiMauro S and Schon EA 1999 Mitochondrial involvement in Alzheimer's disease; *Biochim. Biophys. Acta* 1410 171-182

Bredt D S and Snyder S H 1994 Nitric oxide: a physiologic messenger molecule; *Annu. Rev. Biochem.* 63 175-195

Brunner F 1997 Interaction of nitric oxide and endothelin-1 in ischemia/reperfusion injury of rat heart; *J. Mol. Cell Cardiol.* 29 2363-2374

Buisson A, Margaill I, Callebert J, Plotkine M and Boulu R G 1993 Mechanisms involved in the neuroprotective activity of a nitric oxide synthase inhibitor during focal cerebral ischemia; *J. Neurochem.* 61 690-696

_____, Plotkine M and Boulu R G 1992 The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia. *Br. J. Pharmacol.* 106 766-767

Cada A, de la Torre J C and Gonzalez-Lima F 2000 Chronic cerebrovascular ischemia in aged rats: effects on brain metabolic capacity and behavior; *Neurobiol. Aging* 21 225-233

Calhoun M E, Wiederhold K H, Abramowski D, Phinney A L, Probst A, Sturchler-Pierrat C, Staufenbiel M, Sommer B and Jucker M 1998 Neuron loss in APP transgenic mice; *Nature* 395 755-756

Cash A D, Aliev G, Siedlak S L, Fujioka H, Vinters H V, Tabaton M, Johnson A B, Paula-Barbosa M, Avila J, Jones P K, Smith M A and Perry G 2003 Microtubule reduction in Alzheimer disease and aging is independent of TAU filament formation; *Am. J. Pathol.* (in press)

Castellani R, Hirai K, Aliev G, Drew K L, Nunomura A, Takeda A, Cash AD, Obrenovich M E, Perry G and Smith M A 2002 Role of mitochondrial dysfunction in Alzheimer's disease; *J. Neurosci. Res.* 70 357-360

Cazevieille C, Muller A, Meynier F and Bonne C 1993 Superoxide and nitric oxide cooperation in hypoxia/reoxygenation-induced neuron injury; *Free Radic. Biol. Med.* 14 389-395

Chavez J C, Agani F, Pichiule P and Lamanna J C 2000 Expression of hypoxia-inducible factor-1alpha in the brain of rats during chronic hypoxia; *J. Appl. Physiol.* 89 1937-1942

Chen G, Chen K S, Knox J, Inglis J, Bernard A, Martin S J, Justice A, McConlogue L, Games D, Freedman S B and Morris R G 2000 A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease; *Nature* 408 975-979

Chen H, Chopp M, Zhang Z G and Garcia J H 1992 The effect of hypothermia on transient middle cerebral artery occlusion in the rat; *J. Cereb. Blood Flow Metab.* 12 621-628

Cirillo R, Aliev G, Hornby E J and Prosdocimi M 1994 Endothelium as a therapeutical target in peripheral occlusive arterial diseases: consideration for pharmacological interventions; *Pharmacol. Res.* 29 293-311

Cirillo R, Salvatico E, Aliev G and Prosdocimi M 1992 Effect of cloricromene during ischemia and reperfusion of rabbit hindlimb: evidence for an involvement of leukocytes in reperfusion-mediated tissue and vascular injury; *J. Cardiovasc. Pharmacol.* 20 969-975

Coria F, Castano E, Prelli F, Larrondo-Lillo M, van Duinen S, Shelanski M L and Frangione B 1988 Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis; *Lab. Invest.* 58 454-458

Cormier A, Morin C, Zini R, Tillement J and Lagrue G 2001 *In vitro* effects of nicotine on mitochondrial respiration and superoxide anion generation; *Brain Res.* 900 72-79

Court J A, Lloyd S, Johnson M, Griffiths M, Birdsall N J, Piggott M A, Oakley A E, Ince P G, Perry E K and Perry R H 1997 Nicotinic and muscarinic cholinergic receptor binding in the human hippocampal formation during development and aging; *Brain Res. Dev. Brain Res.* 101 93-105

_____, Martin-Ruiz C, Graham A and Perry E 2000 Nicotinic receptors in human brain: topography and pathology; *J. Chem. Neuroanat.* 20 281-298

Coyle J T and Puttfarcken P 1993 Oxidative stress, glutamate, and neurodegenerative disorders; *Science* 262 689-695

Crawford JG 1998 Alzheimer's disease risk factors as related to cerebral blood flow: additional evidence; *Med. Hypotheses* 50 25-36

Crow J P, Ye Y Z, Strong M, Kirk M, Barnes S and Beckman J S 1997 Superoxide dismutase catalyzes nitration of tyrosines by peroxynitrite in the rod and head domains of neurofilament-L; *J. Neurochem.* 69 1945-1953

Dawson D A 1994 Nitric oxide and focal cerebral ischemia: multiplicity of actions and diverse outcome; *Cerebrovasc. Brain Metab.. Rev.* 6 299-324

Dawson V L, Brahmbhatt H P, Mong J A and Dawson T M 1994 Expression of inducible nitric oxide synthase causes delayed neurotoxicity in primary mixed neuronal-glial cortical cultures; *Neuropharmacology* 33 1425-1430

_____ and Dawson T M 1996 Nitric oxide in neuronal degeneration; *Proc. Soc. Exp. Biol. Med.* 211 33-40

_____, _____, London E D, Bredt D S and Snyder S H 1991 Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures; *Proc. Natl. Acad. Sci. U.S.A.* 88 6368-6371

De Jong G I, De Vos R A, Steur E N and Luiten P G 1997 Cerebrovascular hypoperfusion: a risk factor for Alzheimer's disease? Animal model and postmortem human studies; *Ann. N.Y. Acad. Sci.* 826 56-74

de la Torre J C 1997 Hemodynamic consequences of deformed microvessels in the brain in Alzheimer's disease; *Ann. N. Y. Acad. Sci.* 826 75-91

_____2002a Alzheimer's disease: how does it start? *J. Alzheimer's Disease* 4 497-512

_____ 2002b Alzheimer disease as a vascular disorder: nosological evidence; *Stroke* 33 1152-1162

_____ 2000a Critically attained threshold of cerebral hypoperfusion: the CATCH hypothesis of Alzheimer's pathogenesis; *Neurobiol. Aging* 21 331-342.

_____ and Stefano G B 2000b Evidence that Alzheimer's disease is a microvascular disorder: the role of constitutive nitric oxide; *Brain Res. Rev.* 34 119-136

Dietrich W D, Busto R and Ginsberg M D 1984 Cerebral endothelial microvilli: formation following global forebrain ischemia; *J. Neuropathol. Exp. Neurol.* 43 72-83

Duff K 1998 Transgenic models for Alzheimer's disease; *Neuropathol. Appl. Neurobiol.* 24 101-103

Eliasson M J, Sampei K, Mandir A S, Hurn P D, Traystman R J, Bao J, Pieper A, Wang Z Q, Dawson T M, Snyder S H and Dawson V L 1997 Poly (ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia; *Nat. Med.* 3 1089-1095

Ellis R J, Olichney J M, Thal L J, Mirra S S, Morris J C, Beekly D and Heyman A 1996 Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology* 46 1592-1596

Etiene D, Kraft J, Ganju N, Gomez-Isla T, Gemelli B, Hyman B T, Hedley-Whyte T, Wands J R and de la Monte S M 1998 Cerebrovascular pathology contributes to the heterogeneity of Alzheimer's disease; *J. Alzheimer's Disease.* 1 119-134

Fabricius M and Lauritzen M 1994 Examination of the role of nitric oxide for the hypercapnic rise of cerebral blood flow in rats; *Am. J. Physiol.* 266 H1457-H1464

Faraci F M 1991 Role of endothelium-derived relaxing factor in cerebral circulation: large arteries vs. microcirculation; *Am. J. Physiol.* 261 H1038-H1042

_____ and Brian J E 1994 Nitric oxide and the cerebral circulation; *Stroke* 25 692-703

Fischer E G and Ames 3d A 1972 Studies on mechanisms of impairment of cerebral circulation following ischemia: effect of hemodilution and perfusion pressure; *Stroke* 3 538-542

Fiskum G, Murphy A N and Beal M F 1999 Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases; *J. Cereb. Blood Flow Metab.* 19 351-369

Friston K J and Frackowiak R S 1991 Cerebral function in aging and Alzheimer's disease: the role of PET; *Electroencephalogr. Clin. Neurophysiol.* Suppl 42 355-365

Galle J, Bengen J, Schollmeyer P and Wanner C 1995 Impairment of endothelium-dependent dilation in rabbit renal arteries by oxidized lipoprotein (a). Role of oxygen-derived radicals; *Circulation* 92 1582-1589

Garthwaite J and Beaumont P S 1989 Excitatory amino acid receptors in the parallel fibre pathway in rat cerebellar slices; *Neurosci. Lett.* **107** 151-156

Grammas P, Moore P and Weigel P H 1999 Microvessels from Alzheimer's disease brains kill neurons *in vitro*; *Am. J. Pathol.* **154** 337-342

Granger D N, Benoit J N, Suzuki M and Grisham M B 1989 Leukocyte adherence to venular endothelium during ischemia-reperfusion; *Am. J. Physiol.* **257** G683-G688

Grant D N 1997 Dietary links to Alzheimer's disease; *Alzheimer's Dis. Review* **2** 42-55

Hamada Y, Hayakawa T, Hattori H and Mikawa H 1994 Inhibitor of nitric oxide synthesis reduces hypoxic-ischemic brain damage in the neonatal rat; *Pediatr. Res.* **35** 10-14

Hara H, Huang P L, Panahian N, Fishman M C and Moskowitz M A 1996 Reduced brain edema and infarction volume in mice lacking the neuronal isoform of nitric oxide synthase after transient MCA occlusion; *J. Cereb. Blood Flow Metab.* **16** 605-611

Harik N, Harik S I, Kuo N T, Sakai K, Przybylski R J and Lamanna J C 1996 Time-course and reversibility of the hypoxia-induced alterations in cerebral vascularity and cerebral capillary glucose transporter density; *Brain Res.* 737 335-338

Hellstrom-Lindahl E and Court J A 2000 Nicotinic acetylcholine receptors during prenatal development and brain pathology in human aging; *Behav. Brain Res.* **113** 159-168

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell R L, Atwood C S, Johnson A B, Kress Y, Vinters H V, Tabaton M, Shimohama S, Cash A D, Siedlak S L, Harris P L, Jones P K, Petersen R B, Perry G and Smith M A 2001 Mitochondrial abnormalities in Alzheimer's disease; *J. Neurosci.* **21** 3017-3023

Hock B J Jr and Lamb B T 2001 Transgenic mouse models of Alzheimer's disease; *Trends. Genet.* **17** S7-S12

Hofman A, Ott A, Breteler M M, Bots M L, Slooter A J, van Harskamp F, van Duijn C N, Van Broeckhoven C and Grobbee D E 1997 Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study; *Lancet* **349** 151-154

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F and Cole G 1996 Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice; *Science* **274** 99-102

Huang Z, Huang P L, Panahian N, Dalkara T, Fishman M C and Moskowitz M A 1994 Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase; *Science* 265 1883-1885

Iadecola C 1992 Does nitric oxide mediate the increases in cerebral blood flow elicited by hypercapnia? *Proc. Natl. Acad. Sci. U.S.A* **89** 3913-3916

_____ , Li J, Ebner T J and Xu X 1995 Nitric oxide contributes to functional hyperaemia in cerebellar cortex; *Am. J. Physiol.* **268** R1153-R1162

_____ , Zhang F, Casey R, Clark H B and Ross M E 1996 Inducible nitric oxide synthase gene expression in vascular cells after transient focal cerebral ischemia; *Stroke* **27** 1373-1380

_____ , Zhang F, Niwa K, Eckman C, Turner S K, Fischer E, Younkin S, Borchelt D R, Hsiao K K and Carlson G A 1999 SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein; *Nat. Neurosci.* **2** 157-161

_____ , Zhang F, Xu S, Casey R, Ross M E 1995a Inducible nitric oxide synthase gene expression in brain following cerebral ischemia; *J. Cereb. Blood Flow Metab.* **15** 378-384

_____ , Zhang F, Xu X 1995b Inhibition of inducible nitric oxide synthase ameliorates cerebral ischemic damage; *Am. J. Physiol.* **268** R286-R292

Ignarro L J 1990 Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* **30** 535-560

Iwamoto N, Nishiyama E, Ohwada J and Arai H 1997 Distribution of amyloid deposits in the cerebral white matter of the Alzheimer's disease brain: relationship to blood vessels; *Acta Neuropathol. (Berl.)* **93** 334-340

Jagust W J 1988 Neuroimaging of dementing disorders; in *Neuropathology of Dementing Disorders* pp 25-55 ed. W R Markesbery (London: Amold)

Jakobovits A, Lamb B T and Peterson K R 2000 Production of transgenic mice with yeast artificial chromosomes; *Methods Mol. Biol.* **136** 435-453

Joachim C L, Morris J H and Selkoe D J 1989 Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease; *Am. J. Pathol.* 135 309-319

Kalaria R N 1999 The blood-brain barrier and cerebrovascular pathology in Alzheimer's disease; *Ann. N. Y. Acad. Sci.* **893** 113-125

_____ 2000 The role of cerebral ischemia in Alzheimer's disease; *Neurobiol. Aging* **21** 321-330

_____ and Ballard C 1999 Overlap between pathology of Alzheimer disease and vascular dementia; *Alzheimer Dis. Assoc. Disord.* **13** (S3) S115-S123

Kawai M, Kalaria R N, Harik S I and Perry G 1990 The relationship of amyloid plaques to cerebral capillaries in Alzheimer's disease; *Am. J. Pathol.* 137 1435-1446

Kimoto M, Tsuji H, Ogawa T and Sasaoka K 1993 Detection of NG,NG-dimethylarginine dimethylaminohydrolase in the nitric oxide-generating systems of rats using monoclonal antibody; *Arch. Biochem. Biophys.* 300 657-662

_____, Whitley G S, Tsuji H and Ogawa T 1995 Detection of NG,NG-dimethylarginine dimethylaminohydrolase in human tissues using a monoclonal antibody; *J. Biochem.* **117** 237-238

Kisilevsky R 1998 Amyloid beta threads in the fabric of Alzheimer's disease; *Nat. Med.* **4** 772-773

Kulnane L S and Lamb B T 2001 Neuropathological characterization of mutant amyloid precursor protein yeast artificial chromosome transgenic mice; *Neurobiol. Dis.* 8 982-992

Kulnane L S, Lehman E J, Hock B J, Tsuchiya K D and Lamb B T 2002 Rapid and efficient detection of transgene homozygosity by FISH of mouse fibroblasts; *Mamm. Genome* 13 223-226

Kumar A, Schapiro M B, Haxby J V, Grady C L and Friedland R P 1990 Cerebral metabolic and cognitive studies in dementia with frontal lobe behavioural features; *J. Psychiatr. Res.* **24** 97-109

Kuo Y M, Emmerling M R, Bisgaier C L, Essenburg A D, Lampert H C, Drumm D and Roher A E 1998 Elevated low-density lipoprotein in Alzheimer's disease correlates with brain abeta 1-42 levels; *Biochem. Biophys. Res. Commun.* **252** 711-715

Lafon-Cazal M, Culcasi M, Gaven F, Pietri S and Bockaert J 1993a Nitric oxide, superoxide and peroxynitrite: putative mediators of NMDA-induced cell death in cerebellar granule cells; *Neuropharmacology* 32 1259-1266

_____, Pietri S, Culcasi M and Bockaert J 1993b NMDA-dependent superoxide production and neurotoxicity; *Nature* **364** 535-537

LaManna J C, Boehm K D, Mironov V, Hudetz A G, Hritz M A, Yun J K and Harik S I 1994 Increased basic fibroblastic growth factor mRNA in the brains of rats exposed to hypobaric hypoxia; *Adv. Exp. Med. Biol.* 361 497-502

Lamb B T, Bardel K A, Kulnane L S, Anderson J J, Holtz G, Wagner S L, Sisodia S S and Hoeger E J 1999 Amyloid production and deposition in mutant amyloid precursor protein and presenilin-1 yeast artificial chromosome transgenic mice; *Nat. Neurosci.* 2 695-697

_____ , Call L M, Slunt H H, Bardel K A, Lawler A M, Eckman C B, Younkin S G, Holtz G, Wagner S L, Price D L, Sisodia S S and Gearhart J D 1997 Altered metabolism of familial Alzheimer's disease-linked amyloid precursor protein variants in yeast artificial chromosome transgenic mice; *Hum. Mol. Genet.* 6 1535-1541

Lerman A and Burnett J C Jr 1992 Intact and altered endothelium in regulation of vasomotion; *Circulation* 86 III12-III19

_____ , Hildebrand F L Jr, Margulies K B, O'Murchu B, Perrella M A, Heublein D M, Schwab T R and Burnett J C Jr 1990 Endothelin: a new cardiovascular regulatory peptide; *Mayo Clin. Proc.* 65 1441-1455

_____ , Sandok E K, Hildebrand F L, Burnett J C 1992 Inhibition of endothelium-derived relaxing factor enhances endothelin-mediated vasoconstriction; *Circulation* 85 1894-1898

Lin B, Ginsberg M D and Busto R 2001 Hyperglycaemic but not normoglycemic global ischemia induces marked early intraneuronal expression of beta-amyloid precursor protein; *Brain Res.* **888** 107-116

Lindahl B and Lindahl U 1997 Amyloid-specific heparan sulfate from human liver and spleen; *J. Biol. Chem.* 272 26091-26094

Lovell M A, Xie C and Markesbery W R 1998 Decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease; *Neurology* **511** 562-1566

Lum H and Roebuck K A 2001 Oxidant stress and endothelial cell dysfunction; *Am. J. Physiol. Cell Physiol.* **280** C719-C741

MacAllister R J, Parry H, Kimoto M, Ogawa T, Russell R J, Hodson H, Whitley G S and Vallance P 1996 Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase; *Br. J. Pharmacol.* 119 1533-1540

Markesbery W R 1997 Oxidative stress hypothesis in Alzheimer's disease; *Free Radic. Biol. Med.* 23 134-147

_____ and Carney J M 1999 Oxidative alterations in Alzheimer's disease; *Brain Pathol.* 9 133-146

Marletta M A 1994 Nitric oxide synthase: aspects concerning structure and catalysis; *Cell* 78 927-930

Martin-Ruiz C M, Court J A, Molnar E, Lee M, Gotti C, Mamalaki A, Tsouloufis T, Tzartos S, Ballard C, Perry R H and Perry E K 1999 Alpha4 but not alpha3 and alpha7 nicotinic acetylcholine receptor subunits are lost from the temporal cortex in Alzheimer's disease; *J. Neurochem.* 73 1635-1640

Matz R L, Schott C, Stoclet J C, Andriantsitohaina R 2000 Age-related endothelial dysfunction with respect to nitric oxide, endothelium-derived hyperpolarizing factor and cyclooxygenase products; *Physiol. Res.* 49 11-18

Mecocci P, Beal M F, Cecchetti R, Polidori M C, Cherubini A, Chionne F, Avellini L, Romano G and Senin U 1997 Mitochondrial membrane fluidity and oxidative damage to mitochondrial DNA in aged and AD human brain; *Mol. Chem. Neuropathol.* 31 53-64

_____, MacGarvey U and Beal M F 1994 Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease; *Ann. Neurol.* 36 747-751

_____, MacGarvey U, Kaufman A E, Koontz D, Shoffner J M, Wallace D C and Beal M F 1993 Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain; *Ann. Neurol.* 34 609-616

Meguro K, Blaizot X, Kondoh Y, Le Mestric C, Baron J C and Chavoix C 1999 Neocortical and hippocampal glucose hypometabolism following neurotoxic lesions of the entorhinal and perirhinal cortices in the non-human primate as shown by PET. Implications for Alzheimer's disease; *Brain* 122 1519-1531

Michel T and Feron O 1997 Nitric oxide synthases: which, where, how, and why? *J. Clin. Invest.* **100** 2146-2152

Mironov V, Hritz M A, Lamanna J C, Hudetz A G and Harik S I 1994 Architectural alterations in rat cerebral microvessels after hypobaric hypoxia; *Brain Res.* 660 73-80

Moncada S and Higgs E A 1995 Molecular mechanisms and therapeutic strategies related to nitric oxide; *FASEB J.* 9 1319-1330

_____, Palmer R M and Higgs E A 1991 Nitric oxide: physiology, pathophysiology, and pharmacology; *Pharmacol. Rev.* **43** 109-142

Montine T J, Montine K S and Swift L L 1997 Central nervous system lipoproteins in Alzheimer's disease; *Am. J. Pathol.* 151 1571-1575

Morris S M and Billiar T R 1994 New insights into the regulation of inducible nitric oxide synthesis; *Am. J. Physiol.* **266** E829-E839

Nakashima M N, Yamashita K, Kataoka Y, Yamashita Y S and Niwa M 1995 Time course of nitric oxide synthase activity in neuronal, glial, and endothelial cells of rat striatum following focal cerebral ischemia; *Cell Mol. Neurobiol.* 15 341-349

Nelson J B, Hedican S P, George D J, Reddi A H, Piantadosi S, Eisenberger M A and Simons J W 1995 Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate; *Nat. Med.* 1 944-949

Neve R L and Robakis N K 1998 Alzheimer's disease: a re-examination of the amyloid hypothesis; *Trends Neurosci.* **21** 15-19

Niwa K, Carlson G A and Iadecola C 2000 Exogenous A beta1-40 reproduces cerebrovascular alterations resulting from amyloid precursor protein overexpression in mice; *J. Cereb. Blood Flow Metab.* **20** 1659-1668

Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj E K, Jones P K, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood C S, Petersen R B and Smith M A 2001 Oxidative damage is the earliest event in Alzheimer disease; *J. Neuropathol. Exp. Neurol.* **60** 759-767

_____, _____, Hirai K, Aliev G, Takeda A, Chiba S and Smith M A 1999a Neuronal RNA oxidation in Alzheimer's disease and Down's syndrome; *Ann. N.Y. Acad. Sci.* **893** 362-364

_____, _____, Pappolla M A, Wade R, Hirai K, Chiba S and Smith M A 1999b RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease; *J. Neurosci.* **19** 1959-1964

Octave J N 1995 The amyloid peptide precursor in Alzheimer's disease; *Acta Neurol. Belg.* 95 197-209

Olah L, Wecker S and Hoehn M 2001 Relation of apparent diffusion coefficient changes and metabolic disturbances after 1 hour of focal cerebral ischemia and at different reperfusion phases in rats; *J. Cereb. Blood Flow Metab.* **21** 430-439

Perry E K, Court J A, Lloyd S, Johnson M, Griffiths M H, Spurden D, Piggott M A, Turner J and Perry R H 1996 Beta-amyloidosis in normal aging and transmitter signaling in human temporal lobe; *Ann. N. Y. Acad. Sci.* 777 388-392

Perry G and Smith M A 1998 The case for vascular abnormalities in AD; *Current Res. in AD* 3 181-186

_____, Nunomura A, Hirai K, Takeda A, Aliev G and Smith M A 2000 Oxidative damage in Alzheimer's disease: the metabolic dimension; *Int. J. Dev. Neurosci.* **18** 417-421

_____, Smith M A, McCann C E, Siedlak S L, Jones P K and Friedland R P 1998 Cerebrovascular muscle atrophy is a feature of Alzheimer's disease; *Brain Res.* 791 63-66

Prasad M R, Lovell M A, Yatin M, Dhillon H and Markesbery W R 1998 Regional membrane phospholipid alterations in Alzheimer's disease; *Neurochem. Res.* **23** 81-88

Prelli F, Castano E M, van Duinen S G, Bots G T, Luyendijk W and Frangione B 1988 Different processing of Alzheimer's beta-protein precursor in the vessel wall of patients with hereditary cerebral hemorrhage with amyloidosis-Dutch type; *Biochem. Biophys. Res. Commun.* 151 1150-1155

Price J M, Sutton E T, Hellermann A and Thomas T 1997 beta-Amyloid induces cerebrovascular endothelial dysfunction in the rat brain; *Neurol. Res.* 19 534-538

Radi R, Beckman J S, Bush K M and Freeman B A 1991 Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide; *J. Biol. Chem.* 266 4244-4250

Refolo L M, Pappolla M A, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint G S, Sambamurti K and Duff K 2000 Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model; *Neurobiol. Dis.* 7 321-331

Robinson L J, Weremowicz S, Morton C C and Michel T 1994 Isolation and chromosomal localization of the human endothelial nitric oxide synthase (NOS3) gene; *Genomics* 19 350-357

Roses A D 1995 On the metabolism of apolipoprotein E and the Alzheimer diseases; *Exp. Neurol.* 132 149-156

Sala A, Aliev G M, Rossoni G, Berti F, Buccellati C, Burnstock G, Folco G and Maclouf J 1996 Morphological and functional changes of coronary vasculature caused by transcellular biosynthesis of sulfidopeptide leukotrienes in isolated heart of rabbit; *Blood* 87 1824-1832

Salvatico E, Aliev G M, Novello D and Prosdocimi M 1994 Functional depression of isolated perfused rat heart mediated by activated leukocytes: protective effect of cloricromene; *J. Cardiovasc. Pharmacol.* **24** 638-647

Samadani A F, Dawson T M and Dawson V L 1997 Nitric oxide synthase in model of focal ischemia; *Stroke* 28 1283-1288

Sayre L M, Zelasko D A, Harris P L, Perry G, Salomon R G and Smith M A 1997 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease; *J. Neurochem.* 68 2092-2097

Schulz J B, Matthews R T, Klockgether T, Dichgans J and Beal M F 1997 The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative diseases; *Mol. Cell Biochem.* 174 193-197

Selkoe D J 1994 Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease; *Annu. Rev. Cell Biol.* 10 373-403

Sessa W C 1994 The nitric oxide synthase family of proteins; *J. Vasc. Res.* 31 131-143

Shankar A, Loizidou M, Aliev G, Fredericks S, Holt D, Boulos P B, Burnstock G and Taylor I 1998 Raised endothelin 1 levels in patients with colorectal liver metastases; *Br. J. Surg.* 85 502-506

Shi J, Perry G, Aliev G, Smith M A, Ashe K H and Friedland R P 1999 Serum amyloid P is not present in amyloid beta deposits of a transgenic animal model; *Neuroreport* 10 3229-3232

_____, Perry G, Berridge M S, Aliev G, Siedlak S L, Smith M A, Lamanna J C and Friedland R P 2002 Labelling of cerebral amyloid beta deposits *in vivo* using intranasal basic fibroblast growth factor and serum amyloid P component in mice; *J. Nucl. Med.* 43 1044-1051

_____ , Seyidova D, Perry G, Lamb B T, Neal M L, Siedlak S L, Smith M A, LaManna J C, Friedland R P and Aliev G 2002a Neuronal mitochondria abnormalities in a transgenic mouse model overexpressing amyloid beta; *Abstract in: Society for Neuroscience* 295 20

Skoog I, Kalaria R N and Breteler M M 1999 Vascular factors and Alzheimer disease; *Alzheimer Dis. Assoc. Disord.* S13 S106-S114

Smith J D and Breslow J L 1997 The emergence of mouse models of atherosclerosis and their relevance to clinical research; *J. Intern. Med.* 242 99-109

Smith M A, Perry G, Richey P L, Sayre L M, Anderson V E, Beal M F and Kowall N 1996 Oxidative damage in Alzheimer's; *Nature* 382 120-121

_____, Petot G J and Perry G 1997 Diet and oxidative stress: A novel synthesis of epidemiological data on Alzheimer's disease; *Alzheimer's Disease Review* 2 58-60

_____, Richey Harris P L, Sayre L M, Beckman J S and Perry G 1997a Widespread peroxynitrite-mediated damage in Alzheimer's disease; *J. Neurosci.* 17 2653-2657

_____, Rottkamp C A, Nunomura A, Raina A K and Perry G 2000 Oxidative stress in Alzheimer's disease; *Biochim. Biophys. Acta* 1502 139-144

_____, Sayre L M and Perry G 1996a Is Alzheimer's disease of oxidative stress? *Alzheimer's Disease Review* 1 63-67

_____, Taneda S, Richey P L, Miyata S, Yan S D, Stern D, Sayre L M, Monnier V M and Perry G 1994 Advanced Maillard reaction end products are associated with Alzheimer disease pathology; *Proc. Natl. Acad. Sci. U.S.A.* 91 5710-5714

_____, Vasak M, Knipp M, Castellani R J and Perry G 1998 Dimethylargininase, a nitric oxide regulatory protein, in Alzheimer disease; *Free Radic. Biol. Med.* 25 898-902

Sparks D L 1997 Coronary artery disease, hypertension, ApoE, and cholesterol: a link to Alzheimer's disease? *Ann. N. Y. Acad. Sci.* 826 128-146

_____, Kuo Y M, Roher A, Martin T and Lukas R J 2000 Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations; *Ann. N. Y. Acad. Sci.* 903 335-344

Stamler J S 1994 Redox signaling: nitrosylation and related target interactions of nitric oxide; *Cell* 78 931-936

Stewart-Lee A L, Ferns G A and Anggard E E 1995 Differences in onset of impaired endothelial responses and in effects of vitamin E in the hypercholesterolemic rabbit carotid and renal arteries; *J. Cardiovasc. Pharmacol.* 25 906-913

Stewart P A, Hayakawa K, Akers M A and Vinters H V 1992 A morphometric study of the blood-brain barrier in Alzheimer's disease; *Lab. Invest.* 67 734-742

_____, Isaacs H, Lamanna J C and Harik S I 1997 Ultrastructural concomitants of hypoxia-induced angiogenesis; *Acta Neuropathol. (Berl.)* 93 579-584

Stuehr D J 1997 Structure-function aspects in the nitric oxide synthases; *Annu. Rev. Pharmacol. Toxicol.* 37 339-359

Tagliavini F, Ghiso J, Timmers W F, Giaccone G, Bugiani O and Frangione B 1990 Coexistence of Alzheimer's amyloid precursor protein and amyloid protein in cerebral vessel walls; *Lab. Invest.* 62 761-767

Terzano S, Court J A, Fornasari D, Griffiths M, Spurden D P, Lloyd S, Perry R H, Perry E K and Clementi F 1998 Expression of the alpha3 nicotinic receptor subunit mRNA in aging and Alzheimer's disease; *Brain Res. Mol. Brain Res.* 63 72-78

Thomas A J, Morris C M, Ferrier I N and Kalaria R N 2000 Distribution of amyloid beta 42 *in* relation to the cerebral microvasculature in an elderly cohort with Alzheimer's disease; *Ann. N. Y. Acad. Sci.* 903 83-88

Thorns V, Hansen L and Masliah E 1998 nNOS expressing neurons *in* the entorhinal cortex and hippocampus are affected in patients with Alzheimer's disease; *Exp. Neurol.* 150 14-20

Vinters H V, Miller B L and Pardridge W M 1988 Brain amyloid and Alzheimer disease; *Ann. Intern. Med.* **109** 41-54

Wade J G, Amtorp O and Sorensen S C 1975 No-flow state following cerebral ischemia. Role of increase in potassium concentration in brain interstitial fluid; *Arch. Neurol.* **32** 381-384

Wallace D C 1997 Mitochondrial DNA in aging and disease; *Sci. Am.* **277** 40-47

_____ 1999 Mitochondrial diseases in man and mouse; *Science* **283** 1482-1488

Wang R M F, Pelligrio D A, Baughman V L, Koenig H M and Albrecht R F 1995 The role of neuronal nitric oxide synthase in regulation of cerebral blood flow in normocapnia and hypercapnia in rats; *J. Cereb. Blood Flow Metab.* **15** 774-778

Weinbrecht P, Longmuir I, Knopp J and Mills M 1987 Cerebral microcirculatory changes during exposure to hypoxia; *Adv. Exp. Med Biol.* **215** 259-263

Wever R M F, Luscher T F, Cosentino F and Rabelink T J 1998 Atherosclerosis and the two faces of endothelial nitric oxide synthase; *Circulation* **97** 108-112

Xia Y, Dawson V L, Dawson T M, Snyder S H and Zweier J L 1996 Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury; *Proc. Natl. Acad. Sci. U.S.A.* **93** 6770-6774

Yanagisawa M, Kurihara H, Kimura S, Goto K and Masaki T 1988 A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca^{2+} channels; *J. Hypertens.* **S6** S188-S191