A Unifying Hypothesis of Alzheimer’s Disease. III. Risk Factors

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Normal ageing and Alzheimer’s disease (AD) have many features in common and, in many respects, both conditions only differ by quantitative criteria. A variety of genetic, medical and environmental factors modulate the ageing-related processes leading the brain into the devastation of AD. In accordance with the concept that AD is a metabolic disease, these risk factors deteriorate the homeostasis of the Ca\textsuperscript{2+} – energy – redox triangle and disrupt the cerebral reserve capacity under metabolic stress. The major genetic risk factors (APP and presenilin mutations, Down’s syndrome, apolipoprotein E4) are associated with a compromise of the homeostatic triangle. The pathophysiological processes leading to this vulnerability remain elusive at present, while mitochondrial mutations can be plausibly integrated into the metabolic scenario. The metabolic leitmotif is particularly evident with medical risk factors which are associated with an impaired cerebral perfusion, such as cerebrovascular diseases including stroke, cardiovascular diseases, hypo- and hypertension. Traumatic brain injury represents another example due to the persistent metabolic stress following the acute event. Thyroid diseases have detrimental sequela for cerebral metabolism as well. Furthermore, major depression and presumably chronic stress endanger susceptible brain areas mediated by a host of hormonal imbalances, particularly the HPA-axis dysregulation. Sociocultural and lifestyle factors like education, physical activity, diet and smoking may also modulate the individual risk affecting both reserve capacity and vulnerability. The pathophysiological relevance of trace metals, including aluminum and iron, is highly controversial; at any rate, they may adversely affect cellular defences, antioxidant competence in particular. The relative contribution of these factors, however, is as individual as the pattern of the factors. In familial AD, the genetic factors clearly drive the sequence of events. A strong interaction of fat metabolism and apoE polymorphism is suggested by intercultural epidemiological findings. In cultures, less plagued by the ‘blessings’ of the ‘cafeteria diet – sedentary’ Western lifestyle, apoE4 appears to be not a risk factor for AD. This intriguing evidence suggests that, analogous to cardiovascular diseases, apoE4 requires a hyperlipidaemic lifestyle to manifest as AD risk factor. Overall, the etiology of AD is a key paradigm for a gene-environment interaction. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS — Alzheimer’s disease; risk factors; Down’s syndrome; APP; presenilin; apolipoprotein E; traumatic brain injury; cerebrovascular disease; cardiovascular disease; hypertension; major depression; stress; education; diet; smoking; physical activity; trace metals

THE RISK FACTORS

Various genetic and environmental risk factors have been identified. Case-controlled and community-based studies suggest that a variety of factors contribute to the clinical manifestation of AD, particularly late-onset AD. In the aetiology of AD a paradigmatic gene-environment interaction is manifested.

GENETICS

Many epidemiological studies have shown that a positive family history is a consistent risk factor for AD (Heyman et al., 1984; Mendez et al., 1992). This led to the early notion that AD has a genetic underpinning. So far, mutations in three genes have been identified as causally related to familial early-onset AD (EOAD). These three genes explain most of the highly penetrant EOAD. However, overall these gene mutations represent less than 10 per cent of all AD cases.

Mutations of the amyloid precursor protein gene on chromosome 21

The interest of geneticists in the chromosome 21 (C21) was fuelled by two factors: (i) subjects with Down’s syndrome are trisomic for all or part of
indicating that A\textsubscript{\textbeta} is encoded by a gene which maps to C\textsubscript{21} (Robakis \textit{et al}, 1987; St George-Hyslop \textit{et al}, 1987; Tanzi \textit{et al}, 1987). As result of a meticulous search, allelic heterogeneous mutations in the APP gene just distal to the C-terminus and at the N-terminus of the A\textbeta domain that cosegregate with the AD phenotype have been identified (Chartier-Harlin \textit{et al}, 1991; Goate \textit{et al}, 1991; Murrell \textit{et al}, 1991). Though mutations in the gene for APP account for only a small fraction of cases of early-onset FAD, totalling some 19 families worldwide (Hardy, 1997), the heuristic value of these findings was eminent, indicating that A\textbeta is involved in the etiology of AD.

Pathophysiologically, the mutant APP familial diseases exhibit the AD-typical pattern of an impaired Ca\textsuperscript{2+}–energy–redox triangle. Carriers of APP mutations have a parietotemporal glucose hypometabolism even in asymptomatic individuals (Kennedy \textit{et al}, 1955). Abnormalities of glucose metabolism (Sorbi \textit{et al}, 1995) and Ca\textsuperscript{2+} homeostasis (Tatebayashi \textit{et al}, 1995; Gibson \textit{et al}, 1997) are also found in non-neuronal cells. Intriguingly, some APP mutations alter the signalling-function of APP with regard to G\textsubscript{o} activation (Okamoto \textit{et al}, 1996) (a regulator of Ca\textsuperscript{2+} channels) which may mediate their pro-apoptotic actions (Yamatsuji \textit{et al}, 1996; Giambarella \textit{et al}, 1997). 3-ketoglutarate dehydrogenase activity in brains and fibroblasts is reduced similar to sporadic cases (Gibson \textit{et al}, 1998), corroborating that the pathophysiological processes of familial and sporadic cases share common pathways. APP mutations found in familial AD caused an impaired neuronal plasticity with defective neurite extension and synaptic transmission and thus may already give rise to deficits during brain development (Li \textit{et al}, 1997; Hsia \textit{et al}, 1999).


Mutations of presenilin 1 on chromosome 14 and presenilin 2 on chromosome 1

Chromosomes 14 and 1 harbour loci of genetic mutations, which in an almost fully penetrant fashion cause familial AD with age of onset frequently in the 40s (Hardy, 1997; Mattson \textit{et al}, 1998). The genes encode transmembrane proteins which were called presenilins (PS) (Levy-Lahad \textit{et al}, 1995; Rogaev \textit{et al}, 1995; Sherrington \textit{et al}, 1995). PS mutations account for approximately half of the early-onset familial AD cases (Campion \textit{et al}, 1995; Gomez-Isla \textit{et al}, 1997; Cruts \textit{et al}, 1998). So far a multitude of mutations, with one exception, missense mutations, were identified (Cruts and Van Broeckhoven, 1998). However, one of the mutations which was thought to be causally related to AD was later identified as a non-pathogenic polymorphism (Mattila \textit{et al}, 1998). The ApoE allele does not appear to modify the age of onset (Lendon \textit{et al}, 1997).

Though our knowledge about the physiological

role of PS is still fragmentary, a rough pattern is gradually emerging. (Mattson et al., 1998). Widely expressed in the nervous system, PS have a role in neuronal differentiation (Capell et al., 1977; Hartmann et al., 1997; Shen et al., 1997; Tokuhiro et al., 1998), synaptic plasticity (Parent et al., 1999), cell survival (Shen et al., 1997) and apoptosis (Deng et al., 1996; Wolozin et al., 1996; Kim T-W et al., 1997). PS-1 and -2 are located in the endoplasmic reticulum (ER), Golgi complex and in vesicular structures of the somatodendritic compartment of neurons, predominantly AD-vulnerable regions (Cook et al., 1996; Kovacs et al., 1996; Page et al., 1996; Capell et al., 1997; Culvenor et al., 1997) and are expressed in a coordinated fashion (Thinakaran et al., 1997). In addition to cooperative roles of the PS, opposing roles cannot be ruled out (Mattson et al., 1998). PS are processed endoproteolytically into two fragments (Mercken et al., 1996; Thinakaran et al., 1996). The cleavage is developmentally regulated (Capell et al., 1997; Hartmann et al., 1997), but is not necessarily altered by a PS-1 mutation (Mercken et al., 1996; Okochi et al., 1997). As a 7 transmembrane-spanning receptor-like protein (Dewji and Singer, 1997), PS modulate neuronal excitability, Ca\(^{2+}\) homeostasis and transduction signalling (see below), are upregulated by a variety of metabolic stressors such as ischemia, injury, AD, and trisomy 21 (Cribbs et al., 1996; Ikeda et al., 1998; Tanimukai et al., 1998) and appear to protect against these (Giannakopoulos et al., 1997). For instance, PS-1 expression, although decreased in the AD hippocampus (Takami et al., 1997), is increased in the fraction of NFT-free but decreased in NFT-containing neurons (Giannakopoulos et al., 1997). Downregulation of PS-1 expression results in reduced growth and increases susceptibility to apoptosis (Roperch et al., 1998). Moreover, PS directly interact with APP, presumably in the ER and Golgi (Waragai et al., 1997; Weidemann et al., 1997; Xia et al., 1997), increase the secretion of sAPP (Marambaud et al., 1998) and are required for proper \(\gamma\)-secretase processing of APP (Xia et al., 1998). Thus, PS1 deficiency decreases the turnover of the membrane-associated APP and causes carboxyl-terminal fragments of APP to accumulate (De Strooper et al., 1998; Xia et al., 1998). Another interaction involves intercellular binding of membrane-bound APP and PS, which results in cell–cell adhesion and subsequent, intracellular signalling through tyrosine phosphorylation cascades (Dewji and Singer, 1998).

Presence of the mutant PS-1 gene is associated with reduced perfusion and glucose metabolism of the temporoparietal and cingulate region in affected individuals before and after the development of clinical symptoms (Kennedy et al., 1995; Johnson et al., 1998). Brains of mutant PS-1 carriers exhibit a massively increased A\(\beta\) load and even severe cerebellar pathology (Lemere et al., 1996; Mann et al., 1996; Tamaoka et al., 1998). Plasma levels of A\(\beta\) also were found to be increased (Scheuner et al., 1996). Compatible with a role of PS in differentiation and neuronal plasticity, in transgenic mice mutant PS-1 impairs the neuronal ability to differentiate (Furukawa et al., 1998; Tokuhiro et al., 1998), reduces neurite outgrowth (Furukawa et al., 1998; Dowjat et al., 1999) and alters long-term synaptic plasticity in CA1 hippocampus (Parent et al., 1999).

Mutant PS-1 and PS-2 cause a significant increase of APP expression in fibroblasts (Querfurth et al., 1995), decrease sAPP production and secretion (Ancilio et al., 1997; Marambaud et al., 1998), increase total A\(\beta\) and A\(\beta\)42 intracellular accumulation and secretion in transfected cells and transgenic mice (Borchelt et al., 1996; Duff et al., 1996; Citron et al., 1997; Xia et al., 1998) in an age-dependent manner (Duff et al., 1996; Oyama et al., 1998). Expression of the full-length mutant is required for this capacity, indicating the cooperative interaction of the PS fragments (Tomita et al., 1998). Mutant PS also decreased basal ChAT activity in cultured neuronal cells (Pedersen et al., 1997).

PS have a role in the regulation of apoptosis which may be modulated by their proteolytic cleavage (Kim T-W et al., 1997; Vito et al., 1997). Generation of PS-1 and -2 C-terminal fragments delays apoptosis (Vito et al., 1997; Vezina et al., 1998). PS-1 and -2 mutations, on the other hand, increase cellular vulnerability to apoptosis (Deng et al., 1996; Wolozin et al., 1996; Guo et al., 1998).

Abnormalities of Ca\(^{2+}\) regulation, ion channels, signal transduction pathways, oxidative metabolism and antioxidant status were documented in fibroblasts and lymphoblasts of AD patients carrying mutant PS genes (Ito et al., 1994; Gibson et al., 1996; Latorraca et al., 1998). The role of PS in neuronal Ca\(^{2+}\) homeostasis may be mediated by intrinsic Ca\(^{2+}\) channel properties suggested by homologies to Ca\(^{2+}\) channel domains (Sherrington et al., 1995), by binding and regulating the activity of a G\(_{\alpha}\) protein (Smine et al., 1998), thus modulating Ca\(^{2+}\) currents (Hille, 1994), and by upregulating K\(^{+}\) channel currents (Malin et al., 1998). PS-1 over-
expression potentiates Ca\(^{2+}\) responses (Paul et al., 1997). A missense PS-1 mutation impairs this activity with putative profound sequelae for neuronal excitability (Malin et al., 1998). Presenilin-1 mutations alter Ca\(^{2+}\) signalling, perturb Ca\(^{2+}\) homeostasis and mitochondrial function, enhance oxyradical production in cultured cell lines and synaptosomes and sensitize neurons to apoptosis (Furukawa et al., 1998; Guo et al., 1998; Begley et al., 1999). Both Ca\(^{2+}\) influx and release from ER are involved in the pro-apoptotic action of mutant PS-1 (Guo et al., 1998). The mutant PS-induced events highlight the importance of the ER Ca\(^{2+}\) homeostasis (Meldolesi and Pozzan, 1998) for the cellular vulnerability in conditions of energetic stress (Mattson et al., 1998). This role is corroborated by findings that expression of ER stress proteins, including calreticulin which inhibits the intracellular Ca\(^{2+}\) rise, protect from oxidative stress (Liu H. et al., 1998), that depletion of the ER Ca\(^{2+}\) store prevents neuronal injury and death (Waters et al., 1997) and that inhibition of ER Ca\(^{2+}\)-ATPase can induce apoptosis (Kaneko and Tsukamoto, 1994). The reciprocal association of ER cytochrome c reductase activity, with cholinergic dysfunction and positive correlation with neurofibrillary tangles pathology in AD (Zubenko et al., 1990), points at interrelationships of distinct pathophysiologic events. Furthermore, the pathophysiologic relevance of the compromise of ER Ca\(^{2+}\) homeostasis in ageing is emphasized (Heininger, 1999a). It is speculated that PS, via their K\(^{+}\) channel and G\(_{\text{q}}\) protein regulating properties, fulfil important functions in Ca\(^{2+}\) homeostasis and signalling and by disruption of these physiologic functions in mutant PS vital neuronal metabolic processes are impaired, resulting in abnormal neuronal plasticity and susceptibility to neurodegeneration.

**Down’s Syndrome**

Down’s Syndrome (DS), the most frequent of congenital birth defects, is the consequence of triplication of chromosome 21 (Lejeune et al., 1959). The genetic defect results in developmental anomalies, mental retardation and accelerated ageing, the developmental arrest characterized by reduced brain weight and deficient neuro- and synaptogenesis (Wisniewski et al., 1984; de la Monte and Hedley-Whyte, 1990).

DS is the most common genetic cause of AD. DS is associated with a high incidence of AD with similar prevalence rates to those in normals, but occurring 30–40 years earlier (Lai and Williams, 1989; Visser et al., 1997; Holland et al., 1998). Intellectual deterioration is present in approximately one third of patients at the age of 35 and in 50 per cent in the fifth decade of life with fully-fledged dementia (Fenner et al., 1987; Brugge et al., 1994; Devenny et al., 1996; Oliver et al., 1998) paralleled by a slowing of the electroencephalogram (Soininen et al., 1993). In contrast, the typical neuropathological features of AD, SP and NFT are regularly present in the brains of individuals with DS aged over 35 years (reviewed by Mann, 1993). Thus, in comparison to AD patients, significant differences exist in the correlation of SP density and neuronal loss, atrophy and dementia (Wisniewski and Rabe, 1986; Mann et al., 1987; Oliver et al., 1998). AD pathology may precede, while dementia and brain atrophy follow and correlate closely (Schapiro et al., 1989; Pearson et al., 1990). This phenomenon will be further discussed in part 4 of this series. Similar to AD, middle-aged DS individuals with apoE4 have more impaired and more rapidly declining intellectual functions (Alexander et al., 1997b; Del Bo et al., 1997) and an increased risk of developing AD earlier, while apoE2 seems to protect DS individuals from AD (Prasher et al., 1997; Schupf et al., 1998; Tyrrell et al., 1998). DS individuals exhibit a range of disordered processes of brain metabolism and homeostasis, which put the neurons under metabolic stress during the whole lifespan. While younger DS individuals show either normal or increased values for regional cerebral glucose utilization (Schwartz et al., 1983; Schapiro et al., 1990) and blood flow (Risberg, 1980), an AD-like pattern of reduced cerebral glucose metabolism and blood flow can be detected in DS subjects with AD and often in elderly DS individuals without dementia (Melamed et al., 1987; Schapiro et al., 1988; Azari et al., 1994). Typically, older DS subjects show low glucose metabolism during brain stimulation prior to the evolution of dementia (Pietrini et al., 1997). Elderly DS individuals display reduced neuronal counts in cortex, hippocampus, basal forebrain, locus coeruleus, raphe and ventral tegmentum (reviewed by Mann, 1993). Similarly, changes of neurotransmitter markers for cholinergic, noradrenergic, serotonergic, glutamatergic and GABAergic neurons are equivalent to the changes seen in AD (reviewed by Mann, 1993). These can be detected already in middle-aged DS individuals (Casanova et al., 1985; Godridge et al., 1987; Reynolds and Warner, 1988), including a degeneration of basal forebrain neurons (Casanova et al., 1999).
et al., 1985). In fifth decade DS subjects, degradation and/or rapid synthesis of brain cell membranes may occur prior to neuronal loss and degeneration (Murata et al., 1993). Deficiencies of mitochondrial enzymatic activities were demonstrated in DS platelets (Prince J. et al., 1994). DS individuals show increased systemic levels of biomarkers of oxidative stress (Jovanovic et al., 1988) and neurons display in vitro increased oxygen species and apoptosis (Busciglio and Yankner, 1995). Glycation end products indicating oxidative stress could be detected already in foetal DS brains (Odetti et al., 1998). The cerebral metabolic stress may be aggravated by a hypothyroid state. Notably, increased TSH levels indicating a cerebral thyroid hormone shortage predict a poorer cognitive performance (Bhaumik et al., 1991), while autoimmune thyroiditis is a common finding in DS subjects and appears to be more pronounced in individuals affected by AD (Percy et al., 1990).

As a consequence of the extra C21, expression of APP is increased in ageing Down’s Syndrome brains (Rumble et al., 1989; Oyama et al., 1994; Arai et al., 1997), cortical cultures (Yankner et al., 1998), lymphocytes and fibroblasts (Govoni et al., 1996; Pallister et al., 1997) and serum (Rumble et al., 1989). In contrast to controls, plasma levels of Aβ are increased (Tokuda et al., 1997), soluble Aβ is present in DS brains during the whole lifespan (Teller et al., 1996) and, hence, Aβ deposition could be detected in parahippocampal structures as early as in 8 year-old DS patients (Leverenz and Raskind, 1998). Aβ42 deposition precedes and is more abundant, while Aβ40 is only detectable when first signs of neurodegeneration emerge (Iwatsubo et al., 1995; Kalaria et al., 1996). This gene dosage effect is also present in transgenic mice where the level of neuronal expression of human APP determined the neuroprotective/neurotoxic equilibrium, particularly, in the context of secondary neural injuries (Mucke et al., 1996). In vitro, overexpression of APP has been demonstrated to induce neuronal degeneration and intracellular accumulation of APP derivatives (Yoshikawa et al., 1992). In DS cortical cultures, levels of secreted sAPP and Aβ were reduced, while intracellular Aβ accumulated in vitro and in vivo (Yankner et al., 1998). Overexpression of S-100β that localizes to C21 may also contribute to the accelerated brain ageing and impaired neuronal plasticity (Jørgensen et al., 1990; Whitaker-Azmitia et al., 1997). A decreased neuronal connectivity and synaptogenesis, which manifests as functional disruption of neural circuits, may already be apparent in children and progress in non-demented DS subjects in their thirties (Ferrer and Gullotta, 1990; Takashima et al., 1994). Finally, a precarious immunosenescent status of DS patients may condition the cerebral milieu for the AD-like acute phase response (Griffin et al., 1989; Mehta et al., 1993; Oka and Takashima, 1997).

Trisomy 16 (TS16) is the mouse correlate of human DS. A partial TS16 mouse (the full TS16 mouse is not viable) shows developmental delay, abnormal behaviour compatible with mental retardation, septohippocampal cholinergic degeneration and astrogliosis (Holtzman et al., 1996). TS16 cell lines demonstrate aberrant APP processing and cytoskeleton abnormalities (Williams et al., 1998) and show decreased glutathione levels and increased vulnerability (Bambrick et al., 1995; Stabel-Burrow et al., 1997). Intriguingly, TS16 astroglia elicited a cholinergic deficit when co-cultured with normal neurons from euploid littermates (Nelson et al., 1997).

**Mutations of mitochondrial DNA**

Epidemiological studies suggest an increased risk of late-onset AD in offspring of mothers with AD compared to those of fathers with AD (Heyman et al., 1983; Farrer et al., 1991; Duara et al., 1993; Edland et al., 1996), a finding consistent with maternal transmission patterns in AD. Moreover, advanced maternal age at birth may constitute an independent risk in the offspring (Cohen et al., 1982; Rocca et al., 1991). Mutations in mtDNA were suspected to be causally related since mtDNA is maternally inherited in a non-Mendelian way (Giles et al., 1980). Since mtDNA partly encodes four out of five complexes in the oxidative phosphorylation pathway, mutations in mtDNA may result in defective energy homeostasis (Mattson, 1997). A mutation identified as increased in AD patients affects a mitochondrial tRNA gene (Schollner et al., 1993; Hutchin and Cortopassi, 1995; Egensperger et al., 1997). This is not a highly penetrant mutation (Tysoe et al., 1996) and therefore may have been missed by another group (Wragg et al., 1995). Possible consequences for mitochondrial protein synthesis, particularly for complex I (NADH dehydrogenase), have been suggested (Hutchin and Cortopassi, 1995). An increased sensitivity of cells bearing a variety of mtDNA mutations to Ca++-dependent oxidant stress was shown (Wong and Cortopassi, 1997). Another
report recently demonstrated an increased frequency of a mtDNA 5 kb deletion in AD temporal cortex (Hamblet and Castora, 1997). This type of deletion has been shown previously to be associated with Kearns–Sayre syndrome, a mitochondrial disorder which affects different organ systems. However, as with other mtDNA changes the inherited versus acquired nature remains elusive. mtDNA is exposed to high levels of ROS, but has poor repair mechanism, so that during ageing mtDNA point and length mutations accumulate. The increased oxidative stress associated with AD (Heininger, 1999b) may result in a higher frequency of mutations. A recently reported link between heritable mutations in mtDNA encoding cytochrome oxidases and late-onset AD (Davis et al., 1997) could not be confirmed by others (Hutchin et al., 1997) and appears to be an artifact (Hirano et al., 1997; Wallace et al., 1997). Mitochondrial function may also be affected by mutations of nuclear DNA coding for mitochondrial proteins. A polymorphism of the nuclear gene (on chromosome 14) coding for dihydrolipoyl succinyltransferase (DLST), a core component of the 2-ketoglutarate dehydrogenase complex, may be an independent risk factor for late onset AD (Nakano et al., 1997; Sheu et al., 1999a) and appears to be additive to apoE4 (Sheu et al., 1999a). It has been claimed that up to 50 per cent of chromosome 14-related early-onset familial AD cases may be due to the DLST polymorphism (Sheu and Blass, 1999). Another DLST genotype, on the other hand, may protect against AD (Sheu et al., 1999b). An intriguing finding associated mothers who gave birth to a DS child at a relatively young age (<35 years) with an increased risk for AD (Schupf et al., 1994). Another link was suggested for the birth of a DS child and earlier onset of AD in the mother (Heston et al., 1981). Causal relationships may be due to a shared susceptibility for an accelerated ageing process (Emanuel et al., 1972; Brook et al., 1984; Tarin et al., 1998) or a maternal balanced chromosome rearrangement which predisposes to the trisomy syndrome, but does not give rise to an unfavourable phenotypic effect.

Apolipoprotein E

Apolipoprotein (apo) E, a 34 kDa molecular weight protein, is the product of a single gene on chromosome 19. It exists in three different alleles, ε2, ε3, ε4, coding for proteins apoE2, apoE3, apoE4, respectively, differing from each other by one or two amino acids, only. Apo E plays a critical role in lipid metabolism through its systemic function in chylomicron and very low density lipoprotein (VLDL) transport. In the individual organs, it acts by redistributing lipids between areas of excess and demand for proliferation and repair. In the brain, apoE is synthesized and secreted primarily by astrocytes and microglia (Pitas et al., 1987; Nakai et al., 1996; Stone et al., 1997) and upregulated following neuronal injury and reinnervation (Poirier et al., 1993a). Within the damaged area, apoE scavenges cholesterol and phospholipids from cellular debris and recycles it for membrane remodelling associated with neurite sprouting and synaptic replacement (Poirier et al., 1993a). ApoE appears to be necessary for astrocytic lipoprotein secretion (Fagan et al., 1998). Moreover, apoE is involved in synaptic integrity, stabilizes the neuronal cytoskeleton, regulates interactions between neurons and extracellular matrix and modulates intracellular Ca2+ levels (Mashiah et al., 1996b; Holtzman and Fagan, 1998). The crucial importance of these mechanisms for neuronal development and networking was highlighted by the findings that apoE-deficient mice display severe learning deficits (Oitzl et al., 1997a), and age-dependent reduction of synapses, a complete disruption of the dendritic cytoskeleton (Mashiah et al., 1995), and a disturbed transbilayer distribution of cholesterol and lipid structure of synaptic membranes (Igbavboa et al., 1997). The associated compromised synaptic plasticity (Krugers et al., 1997; Holtzman and Fagan, 1998) can be ameliorated by apoE infusions (Mashiah et al., 1996c) and, in a slice culture, by cholesterol (Teter et al., 1998). In transgenic mice produced by transfer of either apoE3 or apoE4 genes to an apoE knockout background, apoE3 and, to a lower extent apoE4, could ameliorate these neuropathologic changes in vivo (Vienberg et al., 1999) and in a hippocampal slice culture (Teter et al., 1998). Evidence suggests that apoE is a stress protein (Einstein et al., 1995; Maquire et al., 1996) which is expressed after acute brain injury due to global hypoxia/ischemia, hypoglycaemia and status epilepticus (Nicoll et al., 1996).

Recent findings have demonstrated a significant association between the ε4 allele and late onset familial and sporadic AD (Corder et al., 1993; Saunders et al., 1993; Poirier et al., 1993b, Rebeck et al., 1993). The ε4 allele increases the risk and lowers the age of onset distribution, so that patients with an ε4 allele develop AD at an earlier age (Hyman et al., 1996; Blacker et al., 1997). In a population-
based incidence study, apoE4 homozygotes had a more than 10-fold higher risk of dementia compared with E3 homozygotes, and around 20 per cent of dementia cases were attributable to apoE4 (Slooter et al., 1998). ApoE4 may also reduce the age of onset of AD in individuals with DS (Schupf et al., 1996; Prasher et al., 1997). On the other hand, the e2 allele appears to lower the risk and increase the age of onset distribution.

A multitude of mechanistic pathways may jointly contribute to the isoform-specific pathogenic effects of ApoE4:

- ApoE4 accelerates the rate of cognitive decline in nondemented individuals (Reed et al., 1994; Helkala et al., 1995; Blesa et al., 1996; Jonker et al., 1998).
- Clinically normal apoE4 subjects in their 50s and 60s exhibit significant reductions of brain metabolic rates for glucose (Small et al., 1995; Reiman et al., 1996). In AD, however, apoE4 does not appear to be associated with a lower glucose utilization (Corder et al., 1997; Higuchi et al., 1997; Hirono et al., 1998).
- ApoE4 promotes incipient AD pathology in nondemented elders (Warzok et al., 1998). Remarkably, initial neurofibrillary changes can be found in apoE4 individuals as early as in their 30s (Ghebremedhin et al., 1998).
- AD patients with ApoE4 may have a lower brain atrophy in relation to their cognitive status, which was thought to indicate an ApoE4-dependent process which induces neuronal dysfunction before overt brain tissue loss becomes manifest (Yasuda et al., 1998).
- ApoE4 may predispose AD patients to particularly low regional cerebral perfusion (Lehtovirta et al., 1998).
- ApoE4 may be associated with reduced cholinergic function in normal and AD brains (Soininen et al., 1995; Beffert and Poirier, 1996; Allen et al., 1997, not confirmed by Svensson et al., 1997; Jørgensen and Mogensen, 1997), and was reported to be associated with a more pronounced loss of metabolic activity of cholinergic nucleus basalis neurons (Salehi et al., 1998).
- ApoE4 increases amyloid load in the AD brain (e.g. Rebeck et al., 1993; Schmechel et al., 1993; Beffert and Poirier, 1996; Hyman et al., 1996) and DS brain (Hyman et al., 1995). This phenomenon may be related to an isoform-specific binding of apoE4 and Aβ. ApoE4 binds less efficiently to Aβ than apoE2 and 3 (LaDu et al., 1994; Zhou et al., 1996; Aleshkov et al., 1997) and hence may accelerate Aβ fibrillization (Wisniewski et al., 1994), and fail to exert the protective actions of apoE3 to Aβ toxicity (Jordan et al., 1998), respectively.
- ApoE4 inhibits or fails to support neurite outgrowth (Nathan et al., 1994) by a receptor-mediated effect (DeMattos et al., 1998) and this effect may be mediated by blockade of microtubule formation (Pitas, 1996). In the presence of a lipid source, expression of human apoE4 blocks, while apoE3 increases neurite outgrowth from murine neuroblastoma cells (Bellosa et al., 1995).
- ApoE4 binds to microtubule-associated proteins less avidly than apoE3, which may result in isoform-specific effects on PHF and NFT formation (Strittmatter et al., 1994; Huang et al., 1994; Fleming et al., 1996) which, however, do not appear to result in quantitative differences in the AD brains (Hyman et al., 1996).
- ApoE4 aggravates the abnormalities of membrane phospholipid metabolism in AD brains (Klunk et al., 1998).
- ApoE4-complexed Aβ is readily sequestered by capillaries and taken up by the brain in vivo, while complexes with apoE2 or 3 have negligible effects (Martel et al., 1997).
- ApoE isoform specifically has antioxidant activity, apoE4 being less potent than E3 and E2 (Miyata and Smith, 1996), thus rendering CSF more susceptible to oxidative stress (Beisiegel et al., 1997).
- ApoE complexed with Aβ enhances Aβ-induced Ca2+ increases in neurons and astrocytes through P/Q type Ca2+ channels and this effect is isoform-specific, E4 being significantly more effective than E3 (Wustenberg et al., 1997; Muller et al., 1998).
- Similarly, an ApoE fragment, which is also found in human brain, isoform-specifically elevated cytoplasmic Ca2+ in cortical and hippocampal cultured neurons both by mobilization of intracellular and influx of extracellular Ca2+ (Marques et al., 1996; Wang and Gruenstein, 1997; Tolar et al., 1997). Addition of protease inhibitors attenuated the neurotoxicity of apoE since the proteolytic fragment was more neurotoxic than full-length apoE (Marques et al., 1997).
- Finally, ApoE also may have impacts on immunological processes. Isoform-specifically, apoE enhances complement activation by Aβ (McGeer et al., 1997). ApoE genotype appears to be an important determinant of microglial activity.
ApoE4 gene dose significantly increased the number of scattered microglial cells (Saitoh et al., 1997) and markers of microglial activation in AD brains (Eggersperger et al., 1998). On the other hand, apoE3 but not apoE4 blocked activation of microglia by APP metabolites (Barger and Harmon, 1997). ApoE may also modulate the specific immune defence as evidenced by the findings that apoE4 is a risk factor for herpes simplex virus (HSV) type 1 infection (Itzhaki et al., 1997) and that ageing and stressed apoE-deficient mice have increased levels of autoantibodies to neuronal antigens (Zhou et al., 1999).

ApoE isoform-specific effects are not specific for AD. Repeatedly (e.g. Myers et al., 1996; Hofman et al., 1997; Katzman et al., 1997; Marin et al., 1998), but compared to AD less consistently (e.g. Pirttila et al., 1996), apoE4 was also suggested as risk factor for vascular dementia, with the larger studies confirming the association. ApoE4 is associated with an increased risk for cardiovascular morbidity and mortality, also in AD patients (Kosunen et al., 1995; Olichney et al., 1997). ApoE4 genotype modulates the risk for a variety of dementing illnesses and may confer an increased risk for dementia after stroke (Kokmen et al., 1996; Slooter et al., 1997), dementia associated with normal pressure hydrocephalus (Nacmias et al., 1997), for both earlier onset and manifestation of frontal lobe dementia (Minthon et al., 1997; Stevens et al., 1997), for traumatic encephalopathy associated with boxing (Jordan et al., 1997), cognitive decline after cardiac operations (Tardiff et al., 1997), senile plaques in temporal lobe epilepsy (Gouras et al., 1997), HIV-related dementia (Corder et al., 1998), worse outcome after traumatic brain injury (Teasdale et al., 1997; Friedman et al., 1999), mortality after intracerebral hemorrhage (Alberts et al., 1995) and cerebrovascular disease in end-stage renal disease patients (Lim et al., 1997). In Creutzfeldt-Jakob disease (CJD) apoE4 is reported to be a risk factor (Amouyel et al., 1994), while in sporadic amyotrophic lateral sclerosis (ALS) apoE4 was related to an earlier onset, and more severe clinical presentation and prognosis (Mouland et al., 1996). Similarly, apoE4 may be associated with an earlier onset and apoE2 with a later onset of Parkinson’s disease (Zareparsi et al., 1997). In general, the apoE4 genotype is associated with an increased vulnerability to and an impaired recovery from a variety of brain injuries (Roses and Saunders, 1997). In apoE ε4 carriers, this increased disposition to neuronal degeneration was highlighted by the finding of a reduced hippocampal volume as early as in their 40s (Tohgi et al., 1997) and the occurrence of neurofibrillary changes as early as in their 30s (Ghebremedhin et al., 1998).

ApoE, as cholesterol carrier, modulates the production of steroid hormones by which it is regulated itself (Reyland et al., 1991). ApoE regulates glucocorticoid (GC) synthesis (Hammami et al., 1991), but for the time being it can only be speculated about an ApoE isoform-specific regulation of GC production. Remarkably, apoE deficient mice showed a blunted initial stress response with lower GC levels (Gordon et al., 1996; Zhou et al., 1998), but higher baseline levels and stronger GC response upon repetitive stress (Zhou et al., 1998). The complex interrelationship between the two risk factors stress/GC (see below) and apoE is epitomized by the finding that lack of apoE in knockout mice induces a cognitive deficit but protects heterozygous mice from stress-related cognitive impairment and improves cognitive performance in stressed homozygous mice (Grootendorst et al., 1998). ApoE isoform modulates the relative, gender-related risk for familial and sporadic AD (Poirier et al., 1993b; Payami et al., 1996), and the protective action of oestrogen replacement (van Duijn et al., 1996). On the other hand, apoE expression is regulated by oestrogen (Srivastava et al., 1996). ApoE polymorphism-mediated alterations of the steroid hormone balance may underly in part the immunological and metabolic phenomena observed in ageing and AD.

Gender

Repeatedly, gender-specific differences in the prevalence/incidence and remaining lifetime risk of AD have been reported, the life expectancy-corrected incidence in women being higher (Silverman et al., 1994; Seshadri et al., 1997; Gao et al., 1998). In DS, women may also exhibit both a higher incidence of clinical AD and a severe phenotypic expression of AD pathology (Raghavan et al., 1994). According to another study, male DS patients may have an increased risk of AD (Schupf et al., 1998). As multifactorial as the etiopathogenesis of AD may be the determinants of a different sex-related risk. Discussion of these factors clearly deserves a broader frame (Heininger, in preparation). Here, only some general principles will be covered briefly. After correction for selective survival due to a different life expectancy, different odds ratios to contract AD...
may be due to a variety of putative pathophysiological variables with sometimes opposite trends on risk status such as X chromosome-associated allelic associations (Zubenko et al., 1998b), susceptibility to apoE genotype (Payami et al., 1996; Martinez et al., 1998; but see Combarros et al., 1998a), cerebral perfusion (Akiyama et al., 1997; Jones et al., 1998), stress responsivity (Seeman et al., 1995; Kiecolt-Glaser et al., 1997), association of hormonal status and memory performance (Oxenkrug et al., 1989; Seeman et al., 1997), education, and life style. Of high relevance is a different time course of ageing-related hormonal decline, being gradual in men and more abrupt in women (Lamberts et al., 1997). Endocrinologically and metabolically, the menopause elicits a drastic change (Richardson, 1993; Spencer et al., 1997), affecting not only gonadal but also thyroid, somatotropic and adrenal hormone levels substantially (Bottiglioni et al., 1983; Van Cauter et al., 1996; Bernardi et al., 1998). Thus, reproductive senescence predicts cognitive decline in aged female monkeys (Roberts et al., 1997). These changes may be accelerated by surgically-induced menopause, as suggested in humans (Nappi et al., 1999) and rodents (Gibbs, 1998), resulting in an increased incidence and earlier onset of AD (Hong-Goka and Chang, 1997, Nee and Lippa, 1999). As confounding factor, the response of neurons to gonadal steroid deprivation and replacement appears to be sexually dimorphic (Miranda et al., 1999).

**Other genetic influences**

Other genetic factors, though actually less well defined, have been reported to modulate the risk for AD.

A polymorphism in the regulatory region of the apoE gene may be associated with an increased risk of AD independent of the apoE4 allele (Bullido et al., 1998; Lambert et al., 1998). A polymorphism in exon 3 of the low-density lipoprotein receptor-related protein (LRP) gene is a risk factor for late-onset AD, again independent of apoE4-associated risk (Kang et al., 1997; Hollenbach et al., 1998). Since LRP is an apoE receptor, this association as well as another with a lipoprotein lipase mutation (Baum et al., 1999) points further at an abnormality of lipoprotein metabolism in AD. A deletion in the gene of z2-macroglobulin, another ligand of the LRP, may also bear a risk for the manifestation of the disease (Blacker et al., 1998). A dinucleotide microsatellite allele flanking the z2-antichymotrypsin gene may modulate the apoE-related risk (Morgan et al., 1997). Another apolipoprotein, CI, has been reported to confer an increased risk with its A allele (Poduslo et al., 1998).

In keeping with the role of immune mechanisms in the pathophysiology of AD, antigens of the HLA immune recognition system may also modulate the risk. Earlier onset of age is associated with the HLA-A2 allele (Payami et al., 1997; Combarros et al., 1998b; Ballerini et al., 1999). In the absence of apoE4, antigens DR1, 2, or 3 may increase the risk while DR4 or 6 may lower the risk for AD (Curran et al., 1997).

Transferrin C2, a variant of the iron transport protein, appears to be weakly associated with AD, emphasizing the role of oxidative stress in the pathophysiology of the disease (van Rensburg et al., 1995; Namekata et al., 1997).

Other genetic associations include the short variant of the polymorphism within the promoter region of the serotonin transporter gene (Oliveira et al., 1998) and a missense mutation of the neurotrophin-3 gene, which was reported to be more frequent in Japanese AD patients than controls (Kunugi et al., 1998). Frameshift mutations due to inaccurate interpretation of intact genetic information, failure of the RNA surveillance system and translation into +1 proteins have been shown for APP and ubiquitin-B (van Leeuwen et al., 1998). This type of mutation, however, appears to be a more general feature of ageing (Evans et al., 1995).

Additional genomic associations can be expected. A complete genomic screen in families affected with late-onset AD revealed an additional locus on chromosome 12 associated with an increased risk for AD (Pericak-Vance et al., 1997). Further allelic associations were detected in a systemic survey of the human genome (Zubenko et al., 1998a).

**MEDICAL RISK FACTORS**

Between the genetic and environmental risk factors, the medical risk factors occupy an intermediate position, since often they are already the composite result of genetic and environmental factors.

**Traumatic brain injury**

Though inconsistent in individual studies (Gentleman and Roberts 1991; Mayeux et al., 1993; Katzman and Kawas, 1994; Kondo et al., 1994; Rasmusson et al., 1995), a meta-analysis found...
traumatic brain injury (TBI) to be associated with an increased risk of AD (Mortimer et al., 1991). Closed head injury in young adulthood may predict cognitive impairment 50 years later and be associated with a higher AD incidence (Havlik et al., 1998). TBI with loss of consciousness >5 min within the preceding 30 years was associated with AD (Schofield et al., 1997a). Evidence suggests that the risk is highest for head injuries that occurred after age 70 (Mayeux et al., 1993) and, in elderslies 70 years or older, major head injuries predicted an accelerated cognitive decline (Luukinen et al., 1999). Similarly, in individuals with the apoE4 genotype the TBI-related risk may be increased (Mayeux et al., 1995; Nicoll et al., 1995) which, however, was not confirmed by another study (O’Meara et al., 1997). Dementia pugilistica following repeated head blows during boxing shares many behavioural, neurophysiological, neurochemical and pathomorphological features of AD (reviewed by Mendez 1995).

TBI may predispose for AD either by reducing the brain reserve in a single event and/or by inducing a long-term process altering neuronal circuitry, excitability and metabolism. TBI is almost invariably associated with neuronal metabolic stress following secondary ischemia (Jenkins et al., 1989; Siesjo et al., 1995; Martin N.A. et al., 1997), cortical spreading depression (Hossmann, 1996; Mayevsky et al., 1996), and resulting excitotoxic exposure (Faden et al., 1989; Yamikami and McIntosh, 1989; Nilsson et al., 1990). These pathophysiological cascades result in regional brain and mitochondrial Ca\(^{2+}\) elevation, oxidative stress, dysfunction of oxidative phosphorylation and ATP decrease (Nadler et al., 1995; Awasthi et al., 1997; Xiong et al., 1997). Neuronal and axonal injury-induced metabolic stress is associated with increased APP expression as part of an acute phase response to the TBI (Gentleman et al., 1993; Pierce et al., 1996). Activation of invading monocytes/macrophages and resident microglial cells occurs with interleukin-1\(\alpha\) expression which is correlated with neuronal APP upregulation (Griffin et al., 1994; Holmin et al., 1998). Deposits of A\(\beta\) were found in the brains of some 30 per cent of patients with fatal TBI (Roberts et al., 1991; Graham et al., 1995). Notably, the likelihood of A\(\beta\) deposition and neurobehavioural deficits increased with age (Hamm et al., 1992). Possibly, the trauma-associated, glucocorticoid- and prostaglandin-mediated inhibition of macrophage phagocytosis may provide the tissue milieu that is permissive to the accumulation of amyloid deposits (Faist et al., 1996; Heininger, 1999b).

TBI, of even low intensity, puts the neurons under metabolic stress with decreased glucose utilization (Hayes and Dixon 1994; Queen and Feeney, 1996) and metabolic depression (Dietrich et al., 1994). The cholinergic system undergoes profound degenerative changes following TBI. In the human traumatized brain, markers of cholinergic neurotransmission are lost (Murdoch et al., 1998). The loss of cholinergic forebrain neurons after TBI (Leonard et al., 1994; Schmidt and Grady, 1995) leads to a disruption of the septohippocampal and basolateral pathway (DeAngelis et al., 1994; Leonard et al., 1997) which is of utmost importance for cognitive processes. Thus, even in the absence of overt cognitive deficits, mild TBI animals showed an increased sensitivity to anticholinergic challenge which persisted after moderate TBI (Dixon et al., 1995).

According to preclinical, clinical and radiological findings both subacute, but persisting and chronic processes may ensue which compromise neuronal circuitry and function and lay the ground for the susceptibility of TBI patients (and ischemia patients, see below) for AD. Profound and prolonged impairments of cognition occur following experimental TBI and ischemia models (Lyeth et al., 1990; Volpe et al., 1992; Wood et al., 1993; Pierce et al., 1998) and human head trauma (Prigatano and Schachter, 1991; Kay et al., 1992; Carlesimo et al., 1997). In animal models, TBI and even subthreshold mechanical injury to the brain induce persistent neurofilament loss (Posmantur et al., 1994) and cytoskeletal pathology (Kanayama et al., 1996; Takeda et al., 1997). A state of chronic stress is evidenced by chronic APP overexpression (Ciallela et al., 1998). Transient ischemia, which is a routine concomitant of TBI, elicits long-term changes with hyperexcitability in rat neocortex and hippocampus due to down-regulation of inhibitory GABAergic function, disturbance of Ca\(^{2+}\) regulation (Tsubokawa et al., 1992; Akaike, 1995; Luhmann et al., 1995; van den Pol et al., 1996; Yang and Benardo, 1997), progressive neurotransmitter receptor imbalances (Araki et al., 1992), chronic depression of glucose metabolism (Beck et al., 1995), and persistent microglial activation (Hsu et al., 1994) resulting in long-term degenerative changes (Mudrick and Baimbridge, 1989; Onodera et al., 1990; Hsu et al., 1994). Similarly, a single systemic dose of the excitotoxin kainic acid induced long-term accumulation of Ca\(^{2+}\) in the rat hippo-
campus (van den Berg and Gramsbergen, 1993).

Furthermore, transient ischemia leads to iron deposition in the cortex and hippocampus, followed by late-onset and persistent lipid peroxidation resulting eventually in neuronal damage (Kondo et al., 1997). Thus, a chronically progressive degenerative process evolves which leads to ongoing axonal degeneration, increasing cytoskeletal abnormality (Takeda et al., 1997; Pierce et al., 1998), progressive loss of cortical and hippocampal tissue and reactive astrocytosis (Smith D. H. et al., 1997; Pierce et al., 1998). A reduction of thyroid endocrine function after head trauma may contribute to the development of brain pathology (Mocchegiani et al., 1995).

Patients with even mild TBI may exhibit abnormal local cerebral metabolic rates correlating with long-term behavioural and cognitive deficits (Ruff et al., 1994; Gross et al., 1996). Subacutely developing hippocampal volume atrophy correlates with cognitive decline in the chronic recovery phase of TBI (Bigler et al., 1997). As a consequence, TBI in young adulthood exacerbates cognitive decline in the following 30 years (Corkin et al., 1989) and may facilitate the evolution of cognitive impairment and AD 50 years later (Havlik et al., 1998). The no-effect threshold may be very low since, even with mild brain trauma, up to 35 per cent of victims may show cognitive sequelae (Bohnen and Jolles, 1992). Persistent abnormalities of the cholinergic system may be causally related to the cognitive deficits as suggested by the therapeutic benefit of a cholinergic agonist (Cadenas et al., 1994).

Cerebral perfusion

The traditional concept assumes that AD and vascular dementia (VD) are two distinct entities. Due to epidemiological evidence that vascular risk factors which are associated with a reduced cerebral blood flow contribute to the pathophysiology of AD (Crawford, 1996, 1998) this dichotomy has come under scrutiny and has increasingly been questioned (Gold et al., 1998; Stewart, 1998). The commonness of vascular abnormalities in AD (Ellis et al., 1996; Buee et al., 1997; see below) and the rarity of isolated cerebrovascular pathology with dementia at autopsy (Hulette et al., 1997; Nolan et al., 1998) highlight the dubiousness of this differentiation. Thus, it appears as if AD and VD represent theoretical concepts with heuristic value, but that the clinical reality is characterized by mixed pictures with varying proportions of respective pathologies. Accordingly, a pathophysiological approach indicates that dementing illnesses can be regarded as a continuum with AD and VD representing two more or less abstract poles rather than distinct entities (Heininger, in preparation). A more radical concept even assumes that cerebral capillary perfusion deficits may trigger the pathophysiological AD cascade (Richardson, 1997; de la Torre, 1999).

The cerebrovascular pathology of AD

Processes related to cellular metabolic stress play a predominant role in the pathophysiology of AD. As already pointed out, the brain as the metabolically most active organ is vitally dependent on a steady supply of oxygen and oxidizable substrates. It can be expected that any shortage of this supply, related or unrelated to the AD-specific processes, would aggravate the disease-related metabolic stress and may accelerate the progression of the disease.

As early as 1938, an AD-related cerebrovascular pathology was described (Scholz, 1938). Other reports followed (reviewed by Kalaria, 1996). However, with the emphasis on the neurobiological processes and the often forced delimitation from VD, scientific interest in the vascular aspect of the disease diminished. Only recently, has this interest been revived (see vol. 826 of the Annals of the New York Academy of Sciences, which contains a wealth of further reading).

The cerebrovascular bed already undergoes slight changes during ageing (Kalaria, 1996; Buee et al., 1997; De Jong et al., 1997; Moody et al., 1997). These include a decrease in vascular density, number of branches and length of microvessels, luminal narrowing, pericyte degeneration and thickening of the basement membrane. In AD, an aggravation of these changes is frequently observed with both a further decreased density of capillaries and arteriolar multiplications and with coiled, fragmented, and twisted microvessels (Perlmutter and Chui, 1990; Kalaria, 1996; Buee et al., 1994, 1997; Moody et al., 1997). Moreover, capillary deposits and basement membrane thickening as well as loss of endothelial mitochondria are more pronounced (De Jong et al., 1997; Zarrow et al., 1997). It can be assumed that these changes are associated with profound haemodynamic consequences, affecting laminar flow and passage of nutrients through the capillary wall (reviewed by de la Torre, 1997b).

AD is very frequently associated with cerebral amyloid angiopathy. Vascular amyloid contains
fibrillar Aβ (Glenn and Wong, 1984). The segments of blood vessels affected by amyloid angiopathy show increased blood–brain barrier permeability, which appears to be due to the disturbed vessel wall structure (Wisniewski et al., 1997). Evidence suggests that Aβ causes the cerebral amyloid angiopathy and the degeneration of the cerebral microvasculature (Kalaria, 1997). Vascular smooth muscle cells may be the source of Aβ and amyloid fibrils (Frackowiak et al., 1995; Wisniewski et al., 1995) and may facilitate the accumulation of Aβ and the assembly of amyloid fibrils (Van Nostrand et al., 1998).

Soluble Aβ is vasoactive, induces vasoconstriction in vivo and in vitro mediated by endothelin, enhances vasoconstriction induced by phenylephrine or endothelin and is toxic to endothelin–enhances vasoconstriction induced by constriction (Kalaria, 1997). Soluble Aβ may also mediate an auto-amplified inflammatory cascade by upregulating cytokines, expression of adhesion molecules and activators of cellular immune reaction (CD40) in vascular cells (Suo et al., 1998). These properties of Aβ are already evident in the pM range and require a random coil or in part β-sheet structure (Crawford et al., 1998b). ApoE4 more than apoE3 may enhance the vasoactivity of Aβ and thus could contribute to the vascular component of AD as well (Paris et al., 1998b).

Cerebrovascular diseases

Various cardiovascular and cerebrovascular diseases appear to be associated with AD. The pathophysiologic common denominators seem to be both a decreased cerebral perfusion leading to a hypo-metabolic state (de la Torre, 1997a), lesions reducing the reserve capacity of the brain and, as in head trauma, persistent pathophysiologic processes following the acute event (see above).

Acute and chronic cerebrovascular processes may predispose to the dementias and pave the way to AD (Pasquier and Leys, 1997). Multiple cerebral infarcts are frequently present in AD brains (Nagy et al., 1997; Snowdon et al., 1997, Heyman et al., 1998), often without a clinical history of stroke (Heyman et al., 1998). Frequently, stroke patients have reduced cognitive abilities (Tatemichi et al., 1996a; Petrovitch et al., 1998; Zhu et al., 1998a), a faster cognitive decline (Zhu et al., 1998b) and are more prone to develop dementia and also AD (Tatemichi et al., 1994b; Brayne et al., 1998; Pohjasvaara et al., 1998; Zhu et al., 1998a) with adverse impact for prognosis (Tatemichi et al., 1994c; Desmond et al., 1998). Subcortical cerebral infarctions elicit global and regional cerebral glucose hypometabolism, which correlates with cognitive capacities (Kwan et al., 1999). Older age at stroke onset, fewer years of education and concomitant hypoxic-ischemic disorders (e.g. seizures, cardiac arrhythmias, pneumonia) are significant covariates (Tatemichi et al., 1994b; Moroney et al., 1996; Pohjasvaara et al., 1998). Transient ischemic attacks are also a risk factor for accelerating cerebral atrophy, cortico-subcortical perfusion decline and incident dementia (Akiyama et al., 1997; Brayne et al., 1998). In large survey studies, vascular factors were important risk factors for AD (Brayne et al., 1997; Hofman et al., 1997) and a cumulative effect of atherosclerosis, white matter lesions (WML) and apoE4 was seen (Hofman et al., 1997, Skoog et al., 1998a). In other retrospective analyses, cardiovascular and cerebrovascular risk factors were common among AD victims (Tiberghien et al., 1993; Tariska et al., 1997). Certain stroke features, e.g. dysphasia (Pohjasvaara et al., 1998), and particularly lacunar infarcts localized in the basal ganglia, thalamus or deep white matter, predispose to dementia, necessitating fewer neuro-pathologic AD lesions for manifestation of the disease (Tatemichi et al., 1993; Snowdon et al., 1997). Accordingly, AD patients with cerebral infarcts or lacunar lesions display a greater overall severity of dementia (Nagy et al., 1997; Snowdon et al., 1997; Heyman et al., 1998).

A marker for atherosclerotic disease, homocysteine (Frishman et al., 1998), was found elevated among AD patients (Clarke et al., 1998; Lehmann et al., 1999), which further confirms the pathophysiological role of atherosclerosis in AD (Miller, 1999).

During normal ageing, cerebral atrophy and WML increase, paralleled by a decline of cerebral perfusion, particularly after age 60 (Claus et al., 1996, 1998; Akiyama et al., 1997; Meyer et al., 1997). WML found in brain imaging studies, or at autopsy, are associated with cognitive impairments and predispose to cognitive deterioration (Pantoni and Garcia, 1997; Meyer et al., 1997). WML indicate and are caused by cerebral hypoperfusion (reviewed by Pantoni and Garcia, 1997) and correlate with vascular risk factors (Amar et al., 1995) and a history of stroke (Breteler et al., 1994b). Although the tissue may attempt to compensate the reduced blood flow by an increased oxygen extrac-
tion (Yamaji et al., 1997; Tohgi et al., 1998). regions of WML denote areas of decreased levels of energy-rich phosphates (Suppey-Marines et al., 1992). WML are a frequent finding in AD — in neuro-pathological series up to 60 per cent of patients were affected — and are associated with a more rapid clinical progression (Brun and Englund, 1986; Wallin et al., 1989a; Diaz et al., 1991). Of note, WML is almost completely absent in early-onset AD, indicating the differential importance of vascular factors in early- versus late-onset AD (Wallin et al., 1989a). These differences are also reflected by the pattern of membrane phospholipid changes in early- and late-onset AD (Svennerholm and Gottfries, 1994). The pathophysiologic importance for AD of a reduced cerebrovascular perfusion was highlighted by the transient clinical improvement of a patient after receiving an omental transposition which improved cerebral blood flow (Goldsmith, 1997).

In animal models of chronic cerebral hypoperfusion induced by bilateral occlusion of common carotid arteries, certain features of WML and AD can be mimicked. Cognitive performance is reduced and sensitivity of cognitive performance to muscarinic blockade is increased (de la Torre et al., 1996). Importantly, the CVD-associated risk of cognitive decline is increased by apoE4 (Haan et al., 1999). CVD is related to poorer cognitive performance and cognitive decline (Breteler et al., 1994a; Haan et al., 1999). Patients with CVD exhibit accelerated brain ageing with a pattern of hippocampal and cortical interneuronal Aβ immunoreactivity strikingly similar to AD and DS (Sparks, 1996a), and increased microglial activation (Streit and Sparks, 1997), in severe cases pronounced senile plaque formation and less prevalent NFT densities (Sparks et al., 1995; Soneira and Scott, 1996). MI and hypertension are significantly and independently associated with WML (Breteler et al., 1994a). Atrial fibrillation is associated with cognitive impairment (Kilander et al., 1999), dementia (De Pedis et al., 1987) and with AD with cerebrovascular disease (Ott et al., 1997). Two separate animal models of CVD show the same pattern of deficits, the severity corresponding to the decreased cardiac output (Sparks, 1996b).

Hypothension and hypertension

Both hypertension and hypotension have been related to the development of dementia including AD (reviewed by Skoog, 1997; Viitanen and Guo, 1997).

Orthostatic hypotension or low blood pressure is a common finding in organic dementia and AD (Guo et al., 1996; Passant et al., 1997; Pohjasvaara et al., 1998; Skoog et al., 1998b) which, although causing decreased regional blood flow, may not be clinically symptomatic (Passant et al., 1997). Repetitive hypotensive episodes may lead to decreased perfusion pressure of the long penetrating arteries of the deep white matter, induce WML and clinically manifest as cognitive impairment (Raina et al., 1993; Dettmers et al., 1997). In line with these findings, low blood pressure was associated with a reduced white matter blood flow in AD patients (Siennicki-Lantz et al., 1998). Low blood pressure was associated with decreased cognitive performance 9 years later (Glynn et al., 1999). In another longitudinal study, a reduction of systolic blood pressure correlated with cognitive decline (Zhu et al., 1998b). In a large cohort of 85-year-olds a low diastolic and systolic blood pressure correlated with frontal and parietal cortical atrophy. Blood pressure decreased further in the

Cardiovascular disease

Cardiovascular disease (CVD) is associated with AD, leading to a six-fold increased incidence of AD type pathology (Sparks et al., 1990; Stewart, 1998). A history of myocardial infarction (MI) increases the risk of probable AD five-fold in older women (Aronson et al., 1990), while a history of heart attack is a risk factor for incident dementia (Brayne et al., 1998). Importantly, the CVD-associated risk of cognitive decline is increased by apoE4 (Haan et al., 1999). CVD is related to poorer cognitive performance and cognitive decline (Breteler et al., 1994a; Haan et al., 1999). Patients with CVD exhibit accelerated brain ageing with a pattern of hippocampal and cortical interneuronal Aβ immunoreactivity strikingly similar to AD and DS (Sparks, 1996a), and increased microglial activation (Streit and Sparks, 1997), in severe cases pronounced senile plaque formation and less prevalent NFT densities (Sparks et al., 1995; Soneira and Scott, 1996). MI and hypertension are significantly and independently associated with WML (Breteler et al., 1994a). Atrial fibrillation is associated with cognitive impairment (Kilander et al., 1999), dementia (De Pedis et al., 1987) and with AD with cerebrovascular disease (Ott et al., 1997). Two separate animal models of CVD show the same pattern of deficits, the severity corresponding to the decreased cardiac output (Sparks, 1996b).

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demented and correlated with dementia severity, suggesting that low blood pressure in dementia is mainly a secondary phenomenon (Skoog et al., 1998b).

Midlife hypertension was found associated with both AD (Kokmen et al., 1991; Kuusisto et al., 1997; White et al., 1998) and AD with white matter disease (Wallin et al., 1989a). Due to a reduced haemodynamic reserve (Fujii et al., 1990), hypertension may lead to dementia as a result of ischaemic cerebrovascular events (see above), being the most important risk factor for stroke (Strandgaard and Paulson, 1994). Independent of stroke, in prospective studies of elderly nondemented persons, hypertension was associated with excess cognitive decline (Launer et al., 1995; Carmelli et al., 1998; Glynn et al., 1999; Haan et al., 1999; Kilander et al., 1999) and accelerated cortical atrophy and cortical perfusion decline (Akiyama et al., 1997; Strassburger et al., 1997). An animal model of hypertension demonstrated the association of cognitive deficits with degeneration of nicotinic transmission, a marker of ageing and AD (Gattu et al., 1997). In longitudinal studies, systolic and diastolic blood pressure were higher in those individuals who developed dementia and AD later (Skoog et al., 1996; Guo et al., 1997). Notably, the manifestation of dementia appeared to be associated with a decrease in blood pressure (Skoog et al., 1996) which continued to decline with increasing dementia severity (Hogan et al., 1997; Skoog et al., 1998b). This may be a potential source of conflicting data with regard to a blood pressure–cognition relationship (see Viitanen and Guo, 1997). Likewise, cross-sectional and retrospective studies in the elderly indicate that hypertension is associated with cognitive impairment, cognitive decline and cerebral WML (Wilkie and Eisdorfer, 1971; Elias et al., 1993; Breterel et al., 1994b; Strassburger et al., 1997; Carmelli et al., 1998; Kilander et al., 1999), the latter increasing with duration of hypertension (de Leeuw et al., 1998). The diastolic blood pressure appeared to be predictive of cognitive impairment on follow-up (Cacciato et al., 1997). Pathophysiologically, hypertension leads to both a decreased regional glucose utilization, CBF, cerebral oxygen supply and cerebral oxygen consumption in humans and laboratory animals (Wei et al., 1992; Nobili et al., 1993; Fujishima et al., 1995; Salerno et al., 1995). Again, apoE genotype may modulate the hypertension-related risk of AD, isoform-specifically (E4 > E3, but not E2) promoting vasoconstriction (Paris et al., 1998b). In comparison with CVD patients, hypertension accentuates the occurrence of NFT indicating the metabolic stress of neurons (Sparks et al., 1995). Hypertensive rats exhibit a decreased level of polyunsaturated fatty acids in synaptosomal membranes as evidence of a higher level of oxidative stress in these animals compared to normotensive controls (Wei et al., 1987, see below).

Therapeutic control of blood pressure may prevent the cognitive impairment, halt the progression of WML (Fukuda and Kitani, 1995; Cacciato et al., 1997), may halt or even reverse the degenerative changes of cerebral microvasculature (Harper, 1987), and reduce the incidence and progression of AD (Guo et al., 1999). A recent double-blind controlled drug trial investigated the long-term treatment of hypertension with a calcium antagonist (Staessen et al., 1997). After a mean follow-up of 2 years, antihypertensive therapy diminished the incidence of AD by 50 per cent (Forette et al., 1998) confirming the pathophysiological role of hypertension in the manifestation of AD and its therapeutic amenability.

Finally, the blood oxygen transportation capacity is an important variable, which together with the perfusion rate determines the total amount of oxygen delivered to the brain. A decrease of this capacity by anaemia also is associated with an increased risk for dementia (Beard et al., 1997).

Thyroid disease

Heyman et al. (1984) were the first to suggest thyroid disease and hypothyroidism as risk factors for AD in a case-control study. This could not be confirmed by others (Amaducci et al., 1986). In the EURODEM meta-analysis of 11 case-control studies, the risk ratio for hypothyroidism was marginally increased (Breterel et al., 1991). Furthermore, an elevated TSH, indicative of a hypothyroid state, was associated with an increased odds ratio for dementia in a community-based study (Ganguli et al., 1996). Patients with sporadic AD present higher titers for autoantibodies indicating autoimmune thyroid disease (Genovesi et al., 1996). Familial AD kindreds display a significant co-segregation between the presence of thyroid autoantibodies and the development of AD (Ewins et al., 1991). Similarly, in DS patients, a pattern of abnormal thyroid function is common, often associated with autoimmune thyroiditis (Murdoch et al., 1977) and more pronounced in patients with AD manifestations (Percy et al., 1990). Cognitive
HYPOTHESIS OF ALZHEIMER’S DISEASE

Dysfunction in patients with hypothyroidism is common, particularly in older individuals (Whybrow et al., 1969; Jain, 1972; Osterweil et al., 1992) and may not be reversible upon therapy (Clarfield, 1988).

Hypothyroidism impairs mitochondrial respiration (Popovici et al., 1980; Dembri et al., 1983), reduces the activity of enzymes of the TCA cycle and ATP content (Glushakova et al., 1976), decreases the rate of glycolysis (Dimitriadis et al., 1989), renders mitochondrial function more vulnerable to Ca^2+ overload (Thomas et al., 1987) and reduces the fluidity particularly of mitochondrial membranes (Taconi et al., 1991). Neurobiologically, hypothyroidism causes impairment of signal transduction processes, lysosomal dysfunction, dysregulation of neurotransmitter and energy metabolism (Iriuchijima et al., 1991; Sinha et al., 1994), reduces slow axonal transport (Stein et al., 1991) and causes brain region-specific biochemical dysfunction (Ahmed et al., 1993). Thyroid hormones (TH) regulate NGF content and expression of low affinity NGF receptors in adult brain (Calza et al., 1997) and, consequently, neurotrophin receptors were decreased in the hypothyreotic rat brain (Alvarez-Dolado et al., 1994). Persistent reduction in choline acetyltransferase activity in the basal forebrain of the rat was detected after thyroid deficiency during early life (Patel et al., 1987). Given the central role of TH in cholesterol metabolism (Ness, 1991), hypothyroidism may also affect AD development by promoting another risk factor, hypercholesterolaemia (see below).

Hyperthyroidism may also lead to impaired brain mitochondrial energy metabolism, uncoupling of oxidative phosphorylation (Popovici et al., 1980), oxidative stress related to the elevated respiratory rate (Satav and Katyare, 1982; Fernandez and Videla, 1993), reduced mitochondrial Ca^{2+} loading capacity, increased mitochondrial Ca^{2+} cycling (Imberti et al., 1994) and finally, to mitochondrial permeability transition (Castilho et al., 1998). Both hypo- and hyperthyroidism induce low density lipoprotein oxidation (Costanini et al., 1998) and may also increase the tissue susceptibility to oxidative challenge, possibly through impairment of the antioxidant capacity (Venditti et al., 1997). These abnormalities of energy metabolism may underly the reported reduction in hippocampal dendritic spine density in hyperthyroid animals (Gould et al., 1991).

Given the profound effects of thyroid dysfunction on brain tropism and metabolism (Heininger, 1999a), the lack of a stronger association between AD and thyroid disease is hard to reconcile with the mounting evidence that a metabolic deficit plays a key role in the pathophysiology of AD. A possible explanation for this discrepancy may come from an inappropriate definition of hypothyroidism in the aged. In the elderly, subclinical hypothyroidism, characterized by normal free T4 levels and elevated TSH levels, is not uncommon (Drinka and Nolten, 1988). These individuals, though not considered as clinically hypothyroid, have an increased risk to become demented (Ganguli et al., 1996) and thus may disguise a stronger thyroid disease/AD relationship. Moreover, the systemic indices give an insufficient picture of the tissue TH state, particularly in the brain. For instance, in the aged even minimal elevations in serum TSH levels are associated with cardiac dysfunctions (Cooper et al., 1984; Forfar et al., 1985) which can be corrected after normalisation of TSH levels (Ridgway et al., 1981; Cooper et al., 1984). High serum cholesterol and an abnormal pattern of lipoproteins may be sensitive indicators of the tissue level hypothryroid state (Kung et al., 1995; Michalopoulou et al., 1998). The pattern of therapeutic response of lipid levels to T4 replacement suggests that in the aged individual the ‘set point’ of hypothalamic–pituitary–thyroid function may be even in the low normal TSH level range (Franklyn et al., 1993; Michalopoulou et al., 1998). Thus, since the hypothalamic–pituitary–thyroid function can be even in the low normal TSH level range, the pattern of therapeutic response of lipid levels to T4 replacement suggests that in the aged individual the ‘set point’ of hypothalamic–pituitary–thyroid function may be even in the low normal TSH level range.

Depression

The findings from individual case-control studies (e.g. Kokmen et al., 1991) were confirmed by the EURODEM Group meta-analysis suggesting a history of late-onset depression as risk factor, increasing the risk for AD independently of family history (Jorm et al., 1991; Van Duijn et al., 1994). A twin study indicated late-life depression as a risk factor for AD as large as two apoE ε4 alleles (Steifens et al., 1997). In addition, a population-based, prospective study suggested early-onset depression as
another risk factor for dementia (Palsson et al., 1999). A retrospective follow-up study over 11 years identified depressive symptoms in a very high proportion of patients as initial manifestations (Evenhuis, 1997).

A variety of neurobiological, endocrinological and immunological events related to the manifestation of major depression (MD) may predispose the CNS for AD. Mild to moderate depression may result in decline of specific cognitive abilities (Boone et al., 1994; Bassuk et al., 1998). This effect appears to be modulated by education, higher education being a protective factor (Palsson et al., 1999). MD is associated with ventricular dilation (Jacoby et al., 1983; Shima et al., 1984; Kellner et al., 1986; Schlegel et al., 1989) which correlates with cognitive impairment and depressive symptomatology (Kellner et al., 1986; Schlegel et al., 1989). Moreover, correlating with the duration and age of onset of MD, the hippocampus is atrophic (Axelson et al., 1993; Sheline et al., 1996). Patients with late-onset MD show a significantly lower whole brain volume, lower tissue density, higher CSF volume and higher ventricle-brain ratio, suggesting a higher degree of central atrophy than in normal aged controls (Jacoby et al., 1983; Shima et al., 1984; Pantel et al., 1997) and also in comparison to aged patients with early-onset MD (Dahabra et al., 1998). Notably, both late-onset minor and major depression were associated with prefrontal atrophy which correlated with illness severity (Kumar et al., 1998). Other imaging studies detected reduced frontal-temporo-parietal perfusion and increased abnormalities of deep white matter in late onset MD (Lesser et al., 1994; Awata et al., 1998; Ebmeier et al., 1998; Greenwald et al., 1998). These WML were associated with cognition deficits and worse antidepressant response (Hickie et al., 1995; Leuchter et al., 1997; Simpson et al., 1997; Awata et al., 1998). Conversely, the CBF abnormalities were found reversible after successful treatment (Bench et al., 1995; Bonne and Krausz, 1997). Finally, brain functional imaging techniques demonstrated a frontal-temporo-parietal glucose hypometabolism in depressive states (Kumar et al., 1993; Biver et al., 1994; Mayberg, 1994).

Depression is associated with a variety of neuroendocrinological disturbances (reviewed by Nemeroff and Krishnan, 1991; Holsboer, 1995; Plotisky et al., 1995). The HPA axis is dysregulated with elevated plasma cortisol levels which normalize after remission (Steiger and Holsboer, 1997). The dexamethasone suppression test (DST) indicates an impaired HPA axis feedback. Of note, ventricular enlargement and hippocampal atrophy correlated with plasma cortisol, but not impaired DST (Kellner et al., 1983; Schlegel et al., 1989; Axelson et al., 1993), while in elderly depressives the latter correlated with the cognitive impairment (Siegel et al., 1989). Likewise, the hypothalamic–pituitary–thyroid axis is hypofunctional (reviewed by Prange et al., 1987; Holsboer, 1995). The TSH response to TRH is often blunted, the nocturnal TSH rise absent, transthryetin is reduced in CSF (Hatterer et al., 1993; Gorman et al., 1997) and euthyroid sick syndrome may be present. Importantly, the peripheral TSH levels are inversely correlated to the global and regional CBF and glucose metabolism (Marangell et al., 1997). Other hormonal abnormalities are present which are equally reminiscent of AD-related changes (Heininger, 1999b): (1) nocturnal melatonin levels are reduced in patients with MD and related disorders (Clausral et al., 1984; Brown et al., 1987; Kennedy et al., 1989); (2) seven studies documented decreased CSF somatostatin levels (reviewed by Rubinow et al., 1992); (3) insulin resistance is a routine finding (Winokur et al., 1988).

The incidence and severity of the MD-related HPA-axis dysregulation increases with age (Asnis et al., 1981; Nelson et al., 1984). Moreover, the ability for compensatory changes decreases. Thus, in comparison to normal controls, depressed children and adolescents have increased nocturnal melatonin levels (Shafii et al., 1996), while in adult and aged depressed these levels are decreased putatively due to CRF-mediated inhibition of melatonin release (Kellner et al., 1997). The pattern of endocrinological alterations which has been suggested to be causally related to the MD-related cognitive disturbances (Reus, 1984; Rubinow et al., 1984) indicates that the neurons of elderly depressed patients are under metabolic stress. Indeed, in patients with affective disorders, blood cells show a compromise of calcium homeostasis and signalling (and, as a corollary, of energy and antioxidant homeostasis, see Heininger, 1999a), which led to the formulation of the calcium hypothesis of depression (reviewed by Dubovsky, 1994; Heininger et al., 1998).

Not surprisingly, these endocrinological abnormalities have profound effects on the immune system of depressed individuals (reviewed by Irwin, 1995). The cellular immune responsivity is blunted (Darko et al., 1988). On the other hand, the innate immune system is activated, including an acute phase response with increased plasma levels of
complement components, α-1-antichymotrypsin, α-2-macroglobulin (Song et al., 1994a), IL-1 and macrophage activation (Maes et al., 1993; McAdams and Leonard, 1993).

Finally, the pathophysiological and putative aetiological relationships between stress (see below) and MD are manifold (reviewed by Akil and Morano, 1995; Post et al., 1995) and may represent a common detrimental pathway into AD manifestation.

PSYCHOSOCIAL AND LIFESTYLE RISK FACTORS

Psychosocial stress

Stressful life events may be another risk factor for AD (Henderson et al., 1992; Shen, 1992; Bratsun, 1998; Zie et al., 1998). Early clinical observations already indicated the relevance of psychological and social adjustment deficiencies (Wang, 1977). Indirect evidence for the pathophysiological role of lifelong stress exposures may be suggested by the premorbid behaviour of social withdrawal. AD patients reportedly exhibit an increased incidence of personality disorders and premorbid personality traits such as psychosocial inactivity, social introversion, pessimism, submissive behaviour and lack of active coping behaviour (Kokmen et al., 1991; Kondo et al., 1994; Bauer et al., 1995; Motomura et al., 1996; Malinchoc et al., 1997). The intriguing finding of an association of AD with marital status, single individuals having an excess risk (Bickel and Cooper, 1994; Helmer et al., 1999), may have a similar basis due to the premorbid personality and behaviours of singles.

AD patients exhibit also biological variables indicating a role of stress exposure and reactivity in the pathophysiology of AD (see Heininger, 1999b). Corticotropin-releasing factor (CRF) and arginine vasopressin neurons in the paraventricular nucleus of the hypothalamus are involved in the regulation of stress responses. These neurons are activated in ageing and AD (Lucassen et al., 1993, 1994; Raadsheer et al., 1995) and in both chronic stress (Makino et al., 1995; Nakase et al., 1998; Scott and Dinan, 1998) and depression (Raadsheer et al., 1995; Scott and Dinan, 1998), suggesting a continuum of pathophysiological processes (Heinrichs, 1999).

Contemporary stress research emphasizes the importance of the individual appraisal of the stressor. The degree to which the individual can cope with or defend against the stressor determines the individual stress hormone profile and development of stress pathologies (Virgin and Sapolsky, 1997; Bookwala and Schulz, 1998). This variability of coping responses to given stressors, however, makes detection of associations of psychosocial stress exposures with disease risks a cumbersome undertaking. The individual stress vulnerability is modulated very early in life as result of a gene-environment interaction (Bouchard, 1994; Plomin et al., 1994; Heim et al., 1997). In experimental animals, prenatal and postnatal stress increase HPA-axis responsivity, prolong stress-induced corticosterone secretion in later life (Maccari et al., 1995) and impair cognitive performance in vulnerable animals (Lyons et al., 1997; Oitzl et al., 1997b). On the other hand, postnatal maternal care and neonatal handling reduce HPA reactivity, improve cognitive performance and protect the hippocampus from age-related dysfunction and neuronal loss (Meany et al., 1988; Mohammed et al., 1993; Sapolsky 1993; Liu et al., 1997).

A variety of conditions associated with elevated GC levels such as chronic stress, depression (see above) and Cushing’s syndrome have detrimental sequelae for human brain function and structure (Sapolsky, 1996; Keenan and Kuhn, 1999). Exposure to physiologic stress level GC induces impaired memory performance in healthy human adults (Newcomer et al., 1994; Wolkowitz, 1994). In normal aged, GC levels correlate negatively with intellectual performance (Lupien et al., 1994; Seevan et al., 1995, 1997; Kelly et al., 1996) and hippocampal volume (Lupien et al., 1998). Longitudinal and cross-sectional studies indicate a memory-deteriorating effect of chronic GC in humans, elderly individuals being particularly susceptible (Keenan et al., 1996). War veterans with post-traumatic stress disorder or political prisoners who have been tortured suffer from memory deficits (Sutker et al., 1991; Bremner et al., 1993; Basoglu et al., 1994). Cushing’s syndrome is associated with memory impairment, hippocampal atrophy and decreased brain glucose utilization (Starkman et al., 1992; Mauri et al., 1993; Brunetti et al., 1988).

Importantly, the pattern of cognitive impairment suggests premature cognitive ageing (Forget et al., 1996), increases with age and tends to improve after surgical treatment (Mauri et al., 1993). Chronic life stress renders more vulnerable to acute stressful events resulting in an exaggerated sympathetic, neuroendocrine and immune responsivity (Pike et al., 1999).
Monkeys in their natural habitat which experience chronic subordination stress and social isolation exhibit hypercortisolism (Sapolsky et al., 1997). A history of low rank pronounced an ageing-related social withdrawal (Veenema et al., 1997), a behaviour which has been reported to be associated with AD in humans (see above).

In adult life, exposure to acute and chronic social and psychological stressors worsens cognitive performance in laboratory animals (McEwen and Sapolsky, 1995; Blanchard et al., 1995), increases GC levels and impairs hippocampal function and dendritic morphology (Uno et al., 1989; McEwen et al., 1992; McEwen and Magarinos, 1997). Electrophysiological, chronic stress or GC accelerate the development of a pattern of ageing-like changes (Landfield et al., 1978; Kerr et al., 1989; Talmi et al., 1993). The stress or ageing-related changes can be prevented by inhibition of GC synthesis and chronic GC receptor blockade (Bodnoff et al., 1995; Magarinos and McEwen, 1995; Talmi et al., 1996) and mimicked by long-term exposure to GC (Sapolsky et al., 1985; Bodnoff et al., 1995; Endo et al., 1996). The intensity of behavioural and neuroendocrine responses to stressful stimuli determines the rate of ageing and life-span of inbred rodent strains (Gilad and Gilad, 1995). GC, stress and ageing set in motion a vicious cycle leading to infertility in women and impotence in men (Selye, 1976). Stress or ageing-related changes could be aggravated by decreased choline uptake but an upregulation of muscarinic binding sites (Finckstein et al., 1985; Gonzalez and Pazos, 1992). Chronic GC leads to the degeneration of cholinergic neurons in the medial septal area (Tizabi et al., 1989), which may underly the chronic stress-induced enhanced sensitivity to muscarinic antagonists (Kaufe et al., 1998b). An increased stress responsivity is associated with a premature degeneration of the cholinergic septohippocampal pathway and shorter life span (Gilad et al., 1987). Furthermore, chronic GC administration decreases nicotine sensitivity and brain nicotinic receptor binding in mice, the hippocampus, hypothalamic and frontal cortical regions being particularly sensitive (Pauly et al., 1990; Pauly and Collins, 1993). GC also render cholinergic neurons more susceptible to other neurotoxic agents (Hortnagl et al., 1993). The glutamatergic system, known to be activated by GC, may participate in the mediation of the degenerative effects (Michel and Agid, 1995). The neurobiological effects are paralleled by behavioural changes. Ten days' stress induced cholinergic hypersensitivity and resistance to scopolamine-induced amnesia, while 30 days' stress resulted in cholinergic hyposensitivity and learning deficits (Zeribh and Laborit, 1990).

Stress also antagonizes the neurotrophin system. Immobilization and cold stress unbalanced the expression of neurotrophins and their receptors in the rat brain (Foreman et al., 1993; Ueyama et al., 1997). While NGF increased in the cortex and hippocampus, the low-affinity NGF receptor density decreased in the basal forebrain and hippocampus (Foreman et al., 1993; Aloe et al., 1994). The equally disruptive effects of physiological stress level GC on neurotrophin levels and signal transduction has been discussed earlier (Heininger, 1999a).

Stress may also interact with hormonal activities of other endocrinological axes. Lower postmenopausal oestradiol levels are associated with stress variables as are oestradiol levels during the menstrual cycle and chronic stress may even lead to infertility in women and impotence in men (Selye, 1976; Ballinger, 1990). In young animals the stress-related neurobiological changes could be aggravated by castration and prevented by testosterone.
Moreover, oestrogen treatment reversed the ageing-related loss of HPA axis feedback inhibition (Ferrini et al., 1999) corroborating the importance of protective gonadal hormones (Goodman et al., 1996; Heiningr, 1999a). Furthermore, chronic stress may decrease the activity of the hypothalamic–pituitary–thyroid axis, a feature which may be attenuated in ageing (Bauer et al., 1994; Cizza et al., 1995), possibly due to the restricted functional capacity of the axis (Cizza et al., 1992). Insulin-like growth factor 1 (IGF-1) is suppressed in socially subordinate male baboons (Sapolsky and Spencer, 1997) and the GC-mediated suppression of astrogial IGF-1 expression may play a role in the age-related decline of axonal sprouting (Ye et al., 1997; Woods et al., 1998). Finally, neuropeptide Y mRNA has been found suppressed following acute stress (Thorsell et al., 1998). As an implication of these hormonal alterations psychosocial stressors may increase the vulnerability of cellular antioxidant systems to pathological conditions (Toleikis and Godin, 1995). Chronic stress may be directly involved in AD-specific processes in that GC and stress impair the Ca²⁺–energy–redox triangle (Heiningr, 1999a) and enhance Aβ production (Liu J et al., 1996, 1998).

Stressful events have profound effects on immune parameters (Apanius, 1998; Elenkov et al., 1999), depending on the time course of exposures and responses. Chronic stressors decrease lymphocytes and their proliferative response, natural killer cells, monocytes and their phagocytosis (Song et al., 1994b; Kanno et al., 1997) and induce an acute phase-like pattern of cytokine elevations, including IL-1, IL-6 and TNFα (Zhou et al., 1993; Chancellor-Freeland et al., 1995. Connor and Leonard, 1998), cytokines which stimulate the HPA axis (Sweep et al., 1992; Zhou et al., 1993; Raber et al., 1997). Again, the stress-related effects are more pronounced and prolonged in aged rodents (Odio et al., 1987; Lorens et al., 1990; Kanno et al., 1997).

Chronic stress may also affect the vascular component of AD pathophysiology. Acute psychological stress elevates persistently total and lipoprotein-associated cholesterol levels (Servatius et al., 1993; Muldoon et al., 1995) and psychosocial stress factors were associated with an atherogenic serum lipid profile (Vitaliano et al., 1995). Thus, together with an atherogenic lipid profile, an increased stress response predicted a higher progression of cardiovascular atherosclerosis (Barnett et al., 1997). It is speculated that epidemiological studies may underestimate these relationships, since subjects with these risk factors are also at risk to contract other life-threatening diseases such as cancer (Baltrusch et al., 1988) or arteriosclerosis (Ely, 1995) which may prevent them from reaching the age to manifest AD.

**Lack of education and of life-long cognitive activity**

Epidemiology suggests that low education is a risk factor for accelerated memory decline and dementia (Evans et al., 1993; Kondo et al., 1994; Stern et al., 1994; Ott et al., 1995; Brayne et al., 1997; Zhu et al., 1998b; Lyketsos et al., 1999). A diagnostic bias, however, related to the sensitivity of the psychometric instruments and the uniformity of threshold values for low and high educated has to be taken into account (Fratiglioni et al., 1998; Geerlings et al., 1999). A low occupational challenge as further risk factor for AD highlights the equal importance of life-long mental activity as a protective factor (Fratiglioni et al., 1993; Stern et al., 1994; Bonaito et al., 1995; Jorm et al., 1998). Conversely, pre-morbid interests and activities were found reduced in patients with Alzheimer’s disease as compared to age- and sex-matched controls (Shen, 1992; Friedland et al., 1997). According to a ‘use it or lose it’ concept, a lifetime use of the brain by continuing education and learning appears to build up a brain reserve capacity which protects against the losses of ageing and eventual contraction of AD (Swaab, 1991; Timiras, 1995; Mortimer, 1997) and even may prevent cognitive impairment despite a widespread AD pathology (Katzman et al., 1998; Davis et al., 1999). Hence in AD patients with the same level of cognitive impairment, educated individuals exhibit a reduced cerebral metabolism compared with less educated individuals. On the other hand, comparing patients with the same abnormality of cerebral metabolism, the educated have more preserved intellectual abilities (Alexander et al., 1997a). Even during AD the verbal competence, in contrast to non-verbal capacities, differentiates between patients with low and high education (Filley and Cullum, 1997). An intriguing finding supports the reserve capacity concept: head circumference and direct brain volume measurements by MRI identified a small brain volume as a risk factor for AD (Mori et al., 1997; Schofield et al., 1997b). Education, on the other hand, increases brain size (Coffey et al., 1999). An interaction between low education and low socioeconomic
rank may confound the reported association of education and AD (Evans et al., 1997; Mortimer et al., 1998, see above). Since poorer education in general leads to lower socioeconomic status, social subordination stress may be a cofactor strengthening the association of low education with cognitive ageing and, finally, AD (Mortimer et al., 1998). Conversely, individuals at risk for AD are protected independently by higher education and socioeconomic status/lower subordination stress against manifesting the disease (Mortimer et al., 1998). Intriguingly, both neuronal activity and stress exposure/coping ability may affect the same neuroendocrinological substrate. Environmental enrichment in adulthood, like neonatal-handling, resulted in a higher hippocampal GC receptor expression, thus promoting the hippocampal feedback inhibition of HPA-axis activity (Mohammed et al., 1993).

These data are extended by findings that both enriched early experience at weaning, training in adulthood and even in senescence result in changes of cortical thickness, size of synaptic contacts and number of dendritic spines and branching compatible with an enhanced use-induced plasticity (Diamond et al., 1985; Van Gool et al., 1987; Rosenzweig and Bennett, 1996). In rodents, environmental enrichment and neuronal activity upregulate growth factors NGF, BDNF and NT-3 (Lindholm et al., 1994; Lauterborn et al., 1996; Pham et al., 1997). Learning and sensorimotor stimulation also increase somatostatin levels in rat brain (Nilsson et al., 1995). Neurotrophins act preferentially on active neurons which, for their part, upregulate neurotrophin receptor expression (Tonigjori et al., 1997). Importantly, the joint action of neurotrophins and electrical activity is required to promote dendritic arborization in brain cortex (McAllister et al., 1996). Moreover, the activity-dependent secretion of neurotrophins generates further secretion of neurotrophins, thus establishing a positive feedback mechanism which reinforces and stabilizes synaptic connections (Canossa et al., 1997). The neuronal trophic response to activation is supported by astroglial, highly potent activity-dependent neurotrophic factors (ADNF) (Gozes and Brenneman, 1996; Bassan et al., 1999). Thus, environmental enrichment increases the density of dendritic contacts (Bogdanovic et al., 1998), promotes neuronal survival in senescent animals (Kempermann et al., 1998) and prevents the ageing-related loss of synaptic connections observed in sensorially deprived controls (Saito et al., 1994).

An interesting in vitro model shed light on the interrelation of use-dependent neuronal plasticity and Ca\(^{2+}\) homeostasis (Nelson et al., 1990). Previous electric stimulation enhanced and strengthened synaptic connectivity between nerve cells and was able to render the neuronal network resistant against the deleterious action of increased calcium levels.

Lack of physical activity

Lack of physical activity has been identified as a risk factor for AD in a prospective study of 7 years’ duration (Yoshitake et al., 1995; Fujishima et al., 1998). Other case-control studies, using retrospective questionnaires, indicated a reduced pre-morbid physical activity level in dementia and AD patients (Li et al., 1991; Henderson et al., 1992; Kondo et al., 1994; Friedland et al., 1997). A variety of features of physical activity (discussed in part 5 of this series) links these intriguing epidemiological findings to the pathophysiological processes of AD.

Diet

Dietary cholesterol clearly can be defined as risk factor for AD which is modulated by apoE polymorphism. In a prospective study, a high intake of saturated fat and cholesterol was associated with an increased risk of dementia, while fish consumption, an important source of polyunsaturated fatty acids protected against dementia and AD (Kalmijn et al., 1997). Another study suggested an increased daily intake of kcal as risk factor (Petot et al., 1998). Mean total cholesterol and low-density lipoprotein cholesterol levels are significantly higher in AD patients than in elderly controls (Lehtonen and Luotonen, 1986; Giubilei et al., 1990; Czech et al., 1994; Kuo et al., 1998; Sulkava et al., 1998) which correlates with apoE4 status (Czech et al., 1994; Sulkava et al., 1998). Brain cholesterol was also found elevated in AD frontal cortex and in CVD (Sparks, 1997b). The high incidence of hyperlipaemia was equal in individuals with VD and AD (54 per cent versus 56 per cent) (Deplanque et al., 1998) arguing for shared pathophysiological pathways (Heininger in preparation). Dietary fat not only increases cholesterol and triglyceride levels in the serum, but also in the brain (Oner et al., 1991). Higher intake of saturated and monounsaturated fatty acids and cholesterol is associated with worse cognitive function (Ortega et al., 1997) while a diet low in fatty acids and high in fibre is associated with
better cognitive performance and lower mortality (Huijbregts et al., 1998). Serum cholesterol levels correlate negatively with cognitive performance in elders (Desmond et al., 1993; Orenko et al., 1996). In aged individuals plasma cholesterol correlates with the presence of white matter lesions (Breteler et al., 1994b) and hyperlipidemia is a risk factor for accelerated cerebral atrophy and cortical perfusion decline (Akiyama et al., 1997).

Dietary fat can alter membrane lipid composition in rat brain and liver, even without directly affecting serum cholesterol levels (Divakaran and Venkataraman, 1997; Kessler et al., 1985; Huertas et al., 1992; Kelly et al., 1995). Ageing, on the other hand, is associated with profound increases of plasmalemmal, mitochondrial and microsomal membrane cholesterol content and of membrane cholesterol/phospholipid mole ratio in a variety of organs and cell types (Grinna, 1977; Rivnay et al., 1979; Nagy et al., 1983; Kessler et al., 1985; Sugawa et al., 1996). Importantly, the asymmetry of cholesterol distribution between exofacial and cytofacial leaflet of synaptic plasma membranes is increasingly lost with ageing (Igbavboa et al., 1996). These changes correspond to the reduced membrane fluidity and Na⁺,K⁺-ATPase activity and increased age-dependent susceptibility to lipid peroxidation (Rivnay et al., 1979; Nagy et al., 1983; Kessler et al., 1985; Viani et al., 1991; Mecocci et al., 1997). Evidence suggests that the decrease in membrane fluidity and a concomitant inhibition of Ca²⁺ + Mg²⁺-ATPase activity depend both on cholesterol content and on increased lipid peroxidation products (North and Fleischer, 1983; Viani et al., 1991; Choi and Yu, 1995; Wood et al., 1995).

Changes in plasmalemmal cholesterol content have substantial consequences for cellular Ca²⁺ and energy homeostasis. Enrichment of cholesterol and its oxidized derivatives in membranes greatly enhance basal and stimulated Ca²⁺ influx and decrease Ca²⁺ + Mg²⁺-ATPase activity which jointly result in increased cytosolic Ca²⁺ levels in a variety of cell types (Boissonneault and Heiniger, 1985; Gleason et al., 1991; Kutryk et al., 1991; Wood et al., 1995). Moreover, activities of liver and heart mitochondrial Ca²⁺ transport, cytochrome oxidases, ATPases, electron transport chain, and adenine nucleotide translocase are decreased, leading to reduced oxidative phosphorylation and a reduced pool of exchangeable adenine nucleotides (ATP and ADP) (Divakaran and Venkataraman, 1977; Rogers et al., 1980; Cunningham et al., 1981; Kopeikina-Tsiboukidou and Del Constantininos, 1983; Kim et al., 1998; Echegoyen et al., 1993). These changes can also be elicited by diets. A high cholesterol-containing diet decreased mitochondrial cytochrome oxidase activity in the monkey liver (Cunningham et al., 1981) depressed mitochondrial respiration in rat liver (Rogers et al., 1980), lowered coenzyme Q10 and increased hydroperoxides in rabbit hepatocyte mitochondria (Ramirez-Tortosa et al., 1997), and partially uncoupled respiration and decreased Ca²⁺-stimulated respiration in swine myocardial mitochondria (Morrison et al., 1997). An atherogenic diet leads to a 50 per cent decrease in brain Ca²⁺ ATPase activity (Oner et al., 1991). Oxidative stress may accentuate these changes, indicated by the finding that membrane hydroperoxides correlated inversely with mitochondrial coenzyme Q levels (Ramirez-Tortosa et al., 1997). Finally, neurotransmitter transport and receptor-mediated signal transduction is impaired by increasing synaptic cholesterol content, the cholinergic stimulation being particularly vulnerable (Crews et al., 1983; Kelly et al., 1995; Sugawa et al., 1996; Denisova et al., 1998). Cholesterol and oxycholesterol can modulate receptor function by two distinct mechanisms, by changes of membrane fluidity and/or specific molecular interactions (Gimpl et al., 1997). Notably, young animals are relatively resistant to such changes (Kelly et al., 1995; Denisova et al., 1998).

Reduction of mitochondrial bioenergetics and aberrations of cholesterol metabolism reinforce each other. Underutilized citrates due to inhibited decarboxylation are degraded to acetyl CoA which are the source for all endogenous cholesterol, thus driving de novo steroid synthesis (Tanner, 1995). Autochthon de novo synthesis is the overwhelming source of brain cholesterol in a variety of mammals, including man (Spady and Dietschy, 1983; Turley et al., 1996) and is insensitive to feedback inhibition by the endproduct (Zhang et al., 1994). Thus, the increased brain membrane cholesterol may depend in part at least not on dietary cholesterol but on the mitochondrial ageing process itself (Choi and Yu, 1995). Dietary cholesterol, on the other hand, affects brain cholesterol metabolism. Hypercholesterolemia accelerates brain ageing with increased brain cholesterol levels, increased oxidative stress (Sparks, 1997a,b) and microglial activation in rabbits (Streit and Sparks, 1977).

In AD brains membrane defects, characterized by decreased phospholipids and precursors and...
increased degradation products have been documented, suggesting an increased phospholipid degradation (Nitsch et al., 1992). Mitochondrial membrane fluidity was reduced, putatively secondary to oxidative stress (Mecocci et al., 1996a). In contrast to ageing, the membranes in affected AD brain regions, but not from regions not affected, show a decreased cholesterol/phospholipid mole ratio, leading to a reduced membrane bilayer width (Wallin et al., 1998b; Mason et al., 1993). Membrane bilayer stability is decreased in AD mid-temporal cortex, but not cerebellum (Ginsberg et al., 1993).

What may be the mechanisms transforming the excess cholesterol content of ageing membranes into the cholesterol deficit in AD membranes? Lipid peroxidation alters the membranes' lipid composition (Viani et al., 1991). Oxidative stress as well as ageing results in a decreased membrane fluidity and increased cholesterol/phospholipid mole ratio (Ghosh et al., 1993; Urano et al., 1997). The increase in cholesterol may be a cellular defence mechanism to protect the membranes from oxidative damage (Subramanian et al., 1993; Jacobsson-Borin et al., 1994; Joseph et al., 1997). Cholesterol is a weak antioxidant and stabilizer of membrane structure (Gutteridge, 1978; Jacobsson-Borin et al., 1994; Vatassery et al., 1995, 1997; Denisova et al., 1997; Joseph et al., 1997). It is suggested that cholesterol is part of the hierarchical antioxidant system (including melatonin, ascorbate, ubiquinol, vitamin E, thiols like glutathione and polyunsaturated fatty acids) which protect cells from the detrimental actions of oxyradicals (Vatassery et al., 1995; Denisova et al., 1997; Joseph et al., 1997; Urano et al., 1997). Cholesterol is protected by the first line antioxidants (Urano et al., 1997). When this system gets under stress and deficient, cholesterol is oxidized and may get mobilized (Huertas et al., 1992), indicating that membrane cholesterol exchanges with exogenous pools when lipid peroxidation occurs. Thus, when oxidative stress is extensive enough, it finally may result in decreased mitochondrial and microsomal membrane cholesterol content (Huertas et al., 1992) and increased brain synaptic plasma membrane fluidity (van Rensburg et al., 1994; Wood et al., 1995). Quantitatively different degrees of the same pathophysiological process, oxidative stress leading to lipid peroxidation, may therefore induce opposite effects on the target variable, the isoprenoid content of biomembranes. Likewise, mobilization of cholesterol by Aβ may contribute to the AD-related increase in membrane fluidity (Wood et al., 1998). Finally, based on fragmentary evidence, a potential link between APP and cholesterol metabolism and function is outlined. The metabolism of both APP and cholesterol is coupled and influences each other. Brain APP metabolites can be modulated by dietary cholesterol, an effect which needs the presence of apolipoproteins (Howland et al., 1998). Cholesterol increases cellular APP levels, decreases metabolism and release of sAPP (Bodovitz and Klein, 1996; Racchi et al., 1997) and enhances amyloidogenic Aβ production (Mizuno et al., 1998) and amyloid deposition (Sparks, 1997a). A reduced sAPP metabolism is also achieved by lipoproteins (Nadeau et al., 1998). Furthermore, both a disturbed membrane cholesterol and sphingomyelin distribution increase Aβ production (Urmonet et al., 1998), while a depletion of cholesterol inhibits Aβ generation in hippocampal neurons (Simons et al., 1998). On the other hand, Aβ decreases the synthesis of various lipids, particularly cholesterol and phospholipids (Koudinova et al., 1996; Walters and Austen, 1998). Moreover, Aβ inhibits cholesterol esterification (Koudinov et al., 1996a; Liu Y et al., 1998), which may be causal to the decreased plasma cholesterol esterification rate in AD and DS (Lacko et al., 1983; Knebl et al., 1994). Serum LDL and total cholesterol levels correlate with brain Aβ N-42 content in AD patients, again indicating a link between lipid and APP metabolism (Kuo et al., 1998). Thus, cholesterol may induce the generation of its own carrier protein and Aβ and cholesterol may interact in a feedback-cycle, whereby Aβ production is upregulated by cholesterol and downregulates cholesterol synthesis. It is speculated that APP and Aβ, in conjunction with apolipoproteins, may serve as lipid transport proteins to direct lipids, particularly cholesterol to cholesterol domains at synaptic sites and stabilize these structures. APP is developmentally regulated and correlated with synaptogenesis (Moya et al., 1994; Morimoto et al., 1998) and is localized at synaptic plasma membranes (Shigematsu et al., 1992; Shimokawa et al., 1993). During synaptogenesis cholesterol is enriched in synaptic structures (Surchev et al., 1995; Schroeder et al., 1995), presumably to stabilize the synaptic formation and reduce lateral shifts of structural components. Aβ is highly lipophilic and as such is secreted, for instance, by hepatoma cells as an apolipoprotein in association with lipoproteins, transthyretin, phospholipids, triglycerides and cholesterol (Koudinov and Koudinova, 1997). In the plasma and CSF, Aβ is associated with both
the apolipoproteins and lipids of high density lipoproteins (Koudinov et al., 1996b, 1998b; LaDu et al., 1998). Aβ has markedly higher binding affinity for cholesterol than for phosphatidylcholine or fatty acids (Aavdulov et al., 1997). This capacity may endow APP as integral constituent of synapses a stabilizing, synaptic domain building property. In fact, Aβ increases the free cholesterol content of membranes (Liu Y. et al., 1998). Putatively due to this interaction, cholesterol protects neurons from the disruption of Ca²⁺ homeostasis and neurototoxicity effected by Aβ in vitro (Hartmann et al., 1994; Zhou and Richardson, 1996) and hence chronic implants in young and old rat brain of cholesterol- or lipid-embedded Aβ are not neurotoxic in vivo (Clemens and Stephenson, 1992).

Diet may also have effects on activities of hormonal feedback axes. High fat and carbohydrate consumption can elevate basal and stress-induced HPA activity in young and elderly individuals (Tannenbaum et al., 1999, 1997a). In rats, free fatty acids activate the HPA axis as well (Widmaier et al., 1995, Tannenbaum et al., 1997b) and reduce plasma corticosteroid binding globulin (Haourigui et al., 1994), resulting in an overall increase of free GC. Finally, dietary fats and proteins appear to have modulatory effects on the inflammatory acute phase response (reviewed by Hellerstein, 1987) and may thus also affect the immune system component of AD pathophysiology.

Intake of several vitamins have been related to the incidence of cognitive decline and dementia. In a longitudinal and cross-sectional study of 22 years' duration, plasma vitamin A and C levels correlated significantly with various variables of cognitive performance (Perrig et al., 1997). A cross-sectional study suggested a correlation between serum vitamin E level and memory performance (Perkins et al., 1999). A prospective study with a mean 4.3 years' follow-up period indicated a protective effect of high-dose vitamins E and C supplement (Morris et al., 1998). A low vitamin C intake was found associated with poorer cognitive performance (Gale et al., 1996). A cross-sectional study reported reduced plasma levels of vitamins A, C and E in AD patients (Foy et al., 1999). AD patients had lower serum folate and vitamin B₁₂ levels (Clarke et al., 1998). In addition, low folate levels were related to worse cognitive performance, a higher incidence of stroke and dementia (Fioravanti et al., 1997; Ebly et al., 1998; Hassing et al., 1999). Elderlies taking a diet containing more carbohydrate, fibre and vitamins (folate, vitamins C, E, and β-carotenes) show a better cognitive capacity (Ortega et al., 1997). Dietary intake of antioxidants such as vitamins A, C and E may be protective against loss of cognitive function (Masaki et al., 1994; Jama et al., 1996; Paleologos et al., 1998, but see Mendelsohn et al., 1998) and the development of AD (Petot et al., 1998). In vitro, antioxidant vitamins can attenuate Aβ cytotoxicity (Yallampalli et al., 1998). Reduced vitamin B₁₂ levels may be secondary, since they were also found in familial AD (McCaddon and Kelly, 1994). Thiamine (vitamin B₁) deficiency has a role in the aetiology of Wernicke’s encephalopathy of chronic alcoholics, elicits in animals an impaired cerebral oxidative metabolism (reviewed by Hazell et al., 1998) and may be associated with an increased incidence of AD (Pepersack et al., 1999). Further mode of actions linking molecular events with preventive effects will be discussed in part 5 of this series.

Further intriguing evidence associated moderate alcohol drinking with a better cognitive performance (Carmelli et al., 1999) and lower incidence of AD (Orgogozo et al., 1997).

Smoking

In a population-based study, smoking was negatively associated with prevalent AD, over a 3-year follow-up, however, the risk of incident AD was equal between smokers and non-smokers (Wang et al., 1999). A meta-analysis of 19 case-control studies (Lee, 1994) showed a strong protective effect of smoking against contracting AD, a finding which was confirmed by later studies (Van Duijn et al., 1995; Callahan et al., 1996; Hillier and Salib, 1997). In keeping with this notion, neuropathologic studies suggested a diminution of senile plaques and neurofibrillary changes in brains of smokers (Perry and Perry, 1993; Ulrich et al., 1997). However, this effect may be specific for AD only, whereas smoking may be a risk factor for VD (Shaji et al., 1996). Smoking may even carry the risk of an overall increase of dementing illnesses (Prince M. et al., 1994; Cobb et al., 1995; Ott et al., 1998; Carmelli et al., 1999), particularly in the presence of another risk factor for atherosclerotic disease, hypertension (Prince M. et al., 1994). Moreover, there may be a dose effect, other case-control studies suggesting heavy smoking as a risk factor for AD (Shalat et al., 1987; Joya-Pardo et al., 1991; Ott et al., 1998; Merchant et al., 1999). Accordingly, in one case-control study light smoking protected from AD and stronger smoking increased the risk (Wang et al.,
1997), while a longitudinal study suggested a decreased risk among former smokers (Merchant et al., 1999). Smoking is associated with premature menopause (Nilsson et al., 1997), but since early menopause is associated with a significantly increased risk of AD (van Duijn et al., 1994) this could further confound the epidemiological evidence. The argument that the putative smoking effect may be due to a selective survival bias — representing a ‘survival of the fittest’ selection — (Riggs, 1993) was questioned (Van Duijn et al., 1995). Mortality, classification (AD or VD) and selection biases make an evaluation of the association smoking-AD problematic. The inconsistent epidemiological association of smoking and AD may be driven both by the neurobiological benefits of nicotine and the cerebrovascular sequelae of smoking.

The epidemiological data suggesting a protective effect of smoking may have a neurobiological basis. The nicotinergic system is thought to play an important role in cognition (reviewed by Warburton, 1991; Levin and Simon, 1998). Acute or chronic nicotine or nicotine-agonists improve learning and cognitive performance in young and aged animals (Meguro et al., 1994; Arendash et al., 1995; Buccafusco et al., 1996) and human smokers and non-smokers (Pritchard et al., 1992; Hasenfratz and Battig, 1994; Algan et al., 1997). Both an enhanced attention, information processing and storage may contribute to these effects (Warburton, 1991). Aged smokers may evade the ageing-related diminution of interhemispheric electrophysiological coherence (Knott and Harr, 1997). Nicotine enhances hippocampal synaptic transmission (Gray et al., 1996) and glutamatergic synaptic transmission by a presynaptic mechanism (Radcliffe and Dani, 1998) and potentiates evoked single cell activity in aged rat prefrontal cortex (Huitron-Resendiz et al., 1997). Nicotinic agonists upregulate nicotinic receptors (Benwell et al., 1988; Wonnacott, 1990; Yang and Buccafusco, 1994) and chronic nicotine, if initiated before normal age-related decline, even promoted the retention of nicotinic receptor expression in rat hippocampus (Rogers et al., 1998). Moreover, nicotine protects neurons against various excitotoxic stressors (Shimohama et al., 1996; Kihara et al., 1997; Zamani et al., 1997) and NGF deprivation (Yamashita and Nakamura, 1996) and attenuated apoptosis in several non-neural cells through a protein kinase C-dependent mechanism (Wright et al., 1993). The upregulation of various constituents of the neurotrophic factor system such as IGF-1 (Kito et al., 1997), fibroblast growth-factor-2 (Kratz et al., 1997) and NGF receptor (Terry and Clarke, 1994; Buccafusco et al., 1996) expression may contribute to the neuroprotective action.

AD brains display a substantial loss of nicotinic cholinergic receptors at autopsy (Shimohama et al., 1986; Whitehouse et al., 1986; Nordberg and Winblad, 1986) and in vivo (Nordberg et al., 1995). Experimentally, lesioning of the cholinergic basal forebrain cells causes degeneration of their target cells in the hippocampus and cortex (Arendash et al., 1987; Nanri et al., 1997). Administration of nicotine can prevent the cell loss and associated cognitive deficit (Decker et al., 1992; Nanri et al., 1997). Accordingly, smokers may exhibit an improved cognitive performance (Letenneur et al., 1994) and postmortem had a higher density of hippocampal and brain nicotinic binding sites and choline acetyltransferase (Benwell et al., 1988; Perry et al., 1996) than non-smoking controls. This protective potential may be related to the modulation of APP metabolism by promoting the secretory pathway (Kim S. H. et al., 1997) and the blocking of Aβ toxicity (Kihara et al., 1997; Kim S. H. et al., 1997; Miao et al., 1997). Thus, although smoking, due to inherent detrimental effects such as atherosclerosis and premature menopause, is not a feasible protectant against AD, nicotine or nicotinic agonists may have a potential as therapeutic opportunities in AD (Jones et al., 1992; Court and Perry, 1994; Parks et al., 1996). In fact, a small trial with 4 weeks of transcutaneous nicotine treatment suggested an improvement of attentional performance in AD patients (White and Levin, 1999).

ENVIRONMENTAL AGENTS

Numerous trace elements, including aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), selen (Se), and zinc (Zn) have been reported to be imbalanced in AD. A comprehensive coverage of this issue is provided by recent reviews (Markesbery and Ehmann, 1994; Olanow and Arendash, 1994).

A putative role of Al in AD has gained widespread attention but has remained controversial despite intense scientific efforts (Savory et al., 1996). Since the first report of an elevated bulk brain Al content in AD (Crapper et al., 1973, 1976), a multitude of studies both confirmed (e.g. Trapp et al., 1978; Ward and Mason, 1987; Corrigan et al., 1993) and refuted (McDermott et al., 1979; Jacobs et al., 1989; Dedman et al., 1992; Bjertness et al., 1996)
these initial findings. Microprobe studies suggested increased Al in the nuclei or tangle portion of NFT bearing neurons (Perl and Brody, 1980; Good et al., 1992; Yumoto et al., 1996) which could not be confirmed by others (Jacobs et al., 1989; Chalfi et al., 1991; Lovell et al., 1993; Makjanic et al., 1998). The content of Al in senile plaques is similarly controversial (Candy et al., 1986; Landsberg et al., 1992). A role for transferrin in the cerebral uptake of Al has been proposed (Roskams and Connor, 1990) but this was dismissed in a study comparing transferrin Al content in situ in AD and control brains (Dedman et al., 1992). Plasma Al levels were also found increased (Basun et al., 1991).

The public health implications of this issue are related to the environmental exposure to Al in drinking water (McLachlan, 1995). A significant increase in the risk of AD was reportedly associated with an elevated Al content (>111 μg/l) in drinking water in comparison with districts with lower Al (<10 μg/l) exposure (Martyn et al., 1989). In one instance, a regional clustering of dementia mortality was ascribed to a high concentration of Al in the drinking water (Frecker, 1991). Supportive findings were obtained in some (Flaten, 1990; Neri and Hewitt, 1991; McLachlan et al., 1996) but not in all further studies (Wood et al., 1988; Wettstein et al., 1991). Confounding factors may be pH, fluoride, Ca²⁺, and silica content of water, the latter three and neutral pH potentially reducing the risk (Birchall et al., 1989; Edwardson et al., 1993; Forbes and Agwani, 1994; Jacqmin et al., 1994). In a series of papers, increased exposure to Al from drinking water (reviewed by Forbes et al., 1995) and occupational exposure to Al (Rifat et al., 1990; Bast-Pettersen et al., 1994) were associated with impaired cognitive functions. However, in a radiotracer study, the total uptake of Al from drinking water was determined to be less than 1 per cent of the typical daily uptake from food (Pries et al., 1998).

The brain is dependent on a steady supply of Fe for normal function (Gerlach et al., 1994). Since free Fe is toxic, its bioavailability has to be controlled stringently. Consistently, iron levels have been found elevated in hippocampus, nucleus basalis, and cortex of AD brains (Andorn et al., 1990; Jellinger et al., 1990; Dedman et al., 1992; Richardson et al., 1992) but lower in AD plasma (Basun et al., 1991). Importantly, loosely bound Fe which is responsible for free radical reactions in vivo is elevated (Kala et al., 1996) and redox-active Fe and an iron regulatory protein were documented in association with SP and NFT (Smith M.A. et al., 1997). The role of iron transport proteins in the brain accumulation of iron has been indicated (Qian and Wang, 1998). Expression of transferrin, an iron scavenger, was greatly up-regulated in affected AD brain regions (Kawamata et al., 1993; Leveugle et al., 1994). Ferritin, which binds and inactivates extracellular iron, similarly increased in AD brains, associated with reactive microglia and NFT (Grundke-Iqbal et al., 1990; Jellinger et al., 1990; Dedman et al., 1992) and was found almost 10 times higher in the cerebrospinal fluid of patients with AD (Kuiper et al., 1994; Robinson et al., 1997). Both alterations can be regarded as a cellular defence mechanism (Focht et al., 1997). Moreover, Fe binding protein p97 levels are elevated in AD patients’ sera (Kennard et al., 1996) and are associated with reactive microglia in AD brains (Jefferies et al., 1996), while the Fe transport protein transferrin is increased in AD frontal cortex (Loeffler et al., 1995). The transferrin C2 subtype, which has an increased frequency in malfunctions which are associated with formation of free radicals, was found to be increased in AD patients (van Rensburg et al., 1995; Namekata et al., 1997; Van Landeghem et al., 1998). Of note, ageing-related changes of Fe homeostasis include increase of total rat brain Fe and possibly ferritin (Roskams and Connor, 1994). Since Fe accumulates in dependence of energy and oxidative stress (Romlo, 1975; Fujimoto et al., 1982; Castelhau et al., 1998), these alterations of Fe homeostasis indicate a status of cellular stress and damage (Ceccarelli et al., 1995; Wang et al., 1995).

Zn fulfils important functions in neuronal transmission, as a component of catalytic sites of enzymes, as an antioxidant and in a structural capacity (Bettger, 1993; Cuajungco and Lees, 1997). Significant increases and imbalances of Zn in the AD brain tissue and in areas of severe AD histopathology associated with senile plaques have been reported (Thompson et al., 1988; Deibel et al., 1996; Cornett et al., 1998; Lovell et al., 1998). Normal (Hershey et al., 1984; Ehmann et al., 1986) and reduced levels were reported as well (Ward and Mason, 1987). In subcellular compartments of AD brains, Zn was decreased in the nuclear fraction (Wenstrup et al., 1990). These changes, although inconsistent, may indicate oxidative stress, since Zn is readily released from its transport protein under oxidative stress (Maret, 1995), but can reduce oxidative stress by displacing redox-active transition metals such as Fe and Cu and, by binding to thiol groups, protect these from oxidation (Bettger, 1993;
Cuajungco and Lees, 1997). In AD patients, an altered Zn homeostasis was also documented in plasma evidenced by a decreased activity of a carrier peptide (Licastro et al., 1996).

A possible pathophysiological role of Cu deficiency in AD was suggested by Hartmann and Evenson (1992). Cu was reportedly decreased in AD hippocampus (Deibel et al., 1996), but elevated in the rim of senile plaques compared with AD neuropil (Lovell et al., 1998). Ceruloplasmin, the Cu transport protein with both antioxidant and oxidant properties (Fox et al., 1995) was found depleted in the AD temporal gyrus (Connor et al., 1993) but increased in the hippocampus and frontal cortex (Loeffler et al., 1996), and increased in AD CSF (Loeffler et al., 1994), indicating an acute phase-type response.

Childhood exposure to Pb was suggested as a risk factor for AD (Prince, 1998). Pb may be a predictor of cognitive performance, with high blood and bone levels associated with impaired cognition (Payton et al., 1998).

Hg was reported to be increased in AD cortex, nucleus basalis and amygdala and in subcellular fractions of the temporal lobe (Ehmann et al., 1986; Thompson et al., 1988; Wenstrup et al., 1990; Connett et al., 1998). Hg levels were also found increased in AD patients' blood (Basun et al., 1991; Hock et al., 1998). Potential mechanisms of Hg neurotoxic actions and their putative role in AD have been discussed (Markesbery and Ehmann, 1994). For instance, Hg may induce signal transduction deficits as encountered in AD (Pendergrass et al., 1997). Dental amalgam, for instance, is a potential source of Hg (Hahn et al., 1990) and brain Hg levels correlated with the number of amalgam restorations (Eggleston and Nylander, 1987). The number of amalgam fillings, however, was not found associated with a lower cognitive performance (Saxe et al., 1995). Environmental sources other than dental amalgam are suspected (Hock et al., 1998), although not identified.

Se has an important role in the regulation of the general redox balance as part of the antioxidant enzymes Se-glutathione peroxidases (Ursini and Bindoli, 1987; Bettger 1993). Se was found reduced in AD brains (Ward and Mason, 1987; Wenstrup et al., 1990).

Modes of neurotoxic actions of trace elements may include modulation of Ca\(^{2+}\) homeostasis (Nicotera and Rossi, 1993), induction or enhancement of oxygen radical formation and augmentation of A\(\beta\) aggregation. Cellular Ca\(^{2+}\) homeostasis may be disrupted by ions of Al (Anghileri and Thouvenot, 1998; Gandolfi et al., 1998), Cr (Anghileri and Thouvenot, 1998), Cu, Fe (Richardson et al., 1992; Anghileri and Thouvenot, 1998), Hg (Nicotera and Rossi, 1993; Hartmann and Atchison, 1998), and Pb (Hegg and Miletic, 1997). Oxidative stress is a general toxic mechanism of a variety of transition metal ions (Stohs and Bagchi, 1995). For instance, it can be induced by Fe (Halliwell and Gutteridge, 1992; Bondy et al., 1998), Cd (Kumar et al., 1996; Sarkar et al., 1997), Cu (Multhaup, 1997; Bondy et al., 1998), Hg (Lund et al., 1993; Hussain et al., 1997) and Mn (Halliwell, 1984), while Al (Gutteridge et al., 1985; Bondy et al., 1998) may not be able to catalyse the free radical formation but enhance the formation elicited by other trace elements. Similarly, the pro-oxidant action of Cd may be mediated by Fe (Casalino et al., 1997). A\(\beta\) may enhance the trace metal-induced ROS formation (Bondy et al., 1998; Dikalov et al., 1999). Cd, Fe, Hg, and Zn may modulate sAPP formation, presumably at the \(\beta\)-secretase level (Bush et al., 1994b; Bodovitz et al., 1995; Smedman et al., 1997; Hock et al., 1998) and facilitate A\(\beta\) toxicity \textit{in vitro} (Schubert and Chevion, 1995). Moreover, Al, Cu, Fe, and Zn were shown to promote A\(\beta\) aggregation (Mantyh et al., 1993; Bush et al., 1994a; Atwood et al., 1998). Finally Al and Pb may contribute to the neurofibrillary pathology (Nikowitz, 1975; Abdel-Ghany et al., 1993).

**GENETIC DISPOSITION OR ENVIRONMENT?**

The composite risk to develop AD is characterized by an interaction of genetic and environmental factors (van Duijn et al., 1994). The allocation of hierarchical positions has been attempted, rating the risk factors according to their pathophysiological relevance. Twin studies suggest both genetic and environmental risk factors in varying proportions in late-onset AD (Nee et al., 1987; Rapoport et al., 1991; Breitner et al., 1995; Lannfelt et al., 1995; Raiha et al., 1996; Gatz et al., 1997). The modulating role of environmental factors is highlighted by the finding that some monozygotic twin pairs have remained discordant for AD for up to 20 years (Breitner et al., 1995). Twin pairs discordant for AD differed in the level of schooling, a higher level carrying a reduced risk (Raiha et al., 1988). In late-onset familial AD, the influence of non-genetic factors may be even greater and the degree of heterogeneity larger (Lopez-Alberola et
al., 1997). However, these studies, carried out in a shared cultural environment, are inadequate to remove environmental factors due to cultural lifestyles like diet. Clues for such influences could be provided by comparison of epidemiological data from studies in different ethnic groups living in the same environment (Treves et al., 1986; Maestre et al., 1995; Farrer et al., 1996; Holder and Warren, 1998). The relatively low prevalence of AD in developing countries may have ethnic and/or environmental causes (Osuntokun and Kalaria, 1997; Kalaria et al., 1997; Hall et al., 1998; Holder and Warren, 1998). In sporadic AD, studies consistently confirm the biological relevance of the association between apoE4 allele and risk of AD across various ethnic origins living in Western culture environments. However, the association seems stronger in Japanese and weaker in American Africans than in Caucasians (Maestre et al., 1995; Tang et al., 1996; Farrer et al., 1997). In contrast, ethnically similar populations living in different environments such as African Americans and Nigerian Africans exhibit not only different AD prevalences (Hendrie et al., 1995a,b; Osuntokun et al., 1995a; Kalaria et al., 1997; Hall et al., 1998; Holder and Warren, 1998). The finding that apoE3 is not associated with higher plasma cholesterol levels (Sandholzer et al., 1995). It was suggested (Sandholzer et al., 1995) that only if exposed to a Western lifestyle with its hypercholesterolaemic diet (Fincham et al., 1987) and lack of physical exercise inherent individual susceptibilities may manifest as burden to develop late-onset disorders such as atherosclerosis and AD (Kamboh et al., 1995). The same conclusion is suggested from studies investigating the prevalence of AD in Old Order Amish, a conservative Christian group in North America, which shuns the modern Western lifestyle. Here, a reduced prevalence of cognitive impairment and AD (Johnson et al., 1997) does not appear to be due to a reduced apoE4 frequency (Pericak-Vance et al., 1996; Holder and Warren, 1998). In this population, which has also a lower cardiovascular mortality (Hamman et al., 1981), a low serum cholesterol, despite a diet high in total and unsaturated fat and cholesterol, is thought to be due to vigorous physical activity (Glick et al., 1998). It is inferred that these examples highlight the interdependence of genetic and environmental prerequisites: apoE4 confers a risk of AD if coincident with increased cholesterol levels (Czech et al., 1994; Jarvik et al., 1995; Chandra and Pandav, 1998; Sulkava et al., 1998) due to certain environmental factors such as high dietary fat intake, psychosocial stressors and lack of physical activity. The cross-cultural epidemiological data clearly relativate the multiple pathophysiological and clinical abnormalities elicited by apoE4 (see above), as of minor functional relevance in an organism which lacks abundant cholesterol supply. Another intriguing genetic–environmental link associated apoE4 with HSV1 infection (Itzhaki et al., 1997; Matsui et al., 1998). Neither factor alone increased the risk for AD, however, the combination strongly correlated with AD brain pathology (Itzhaki et al., 1997). However, evidence suggests that the expression of HSV DNA may be simply an indicator of an underlying immune response deficit related to an HPA axis dysfunction (Dobbs et al., 1993; Sawiris et al., 1994; Noisakran et al., 1998). The finding that apoE4 is also a risk factor for herpes labialis (Lin et al., 1998) may further hint at an association of apoE genotype and immune dysfunction, possibly secondary to an HPA axis dysregulation.

In the familial type AD, genetic factors clearly are of primary importance though even here a less than 100 per cent penetrance (Rossor et al., 1996) and a wide variation in age at onset (Bird et al., 1996; Lopera et al., 1997) point at additional genetic and environmental modulators. These genetic fac-
tors were indicated by an Israeli study showing a significantly higher incidence of early-onset AD in European or American immigrants, than in African or Asian immigrants (Treves et al., 1986). On the other hand, in late-onset FAD, a high degree of phenotypic heterogeneity within families which was even more pronounced between families, was attributed to non-genetic influences (Lopez-Alberola et al., 1997).

In an individual patient a unique combination of genetic, biographic and sociocultural factors may act together causing the many etiologies: one pathogenesis riddle of AD. However, above all these aetiological considerations, the overwhelming importance of ageing as the most significant risk factor should be borne in mind.

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