A Unifying Hypothesis of Alzheimer’s Disease.
II. Pathophysiological Processes

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The age-related loss of hormonal balance puts the homeostatic calcium–energy–antioxidant triangle under stress. The energetic compromise enhances $\beta$-amyloid protein formation and tau protein hyperphosphorylation, both cellular stress responses, which may give rise to diffuse plaque and neurofibrillary tangle formation during normal ageing. Facilitated by a multitude of risk factors (discussed in part III of this series), the age-related processes lead into the pathophysiological events of Alzheimer’s disease (AD). In AD, the hormonal imbalance is further aggravated with progressively detrimental sequelae for the calcium–energy–redox homeostasis and for both amyloid protein precursor and tau protein metabolism. The immune system as an integral component of the neuroendocrine immunological network is subject to the deteriorating endocrinological balance and displays a cellular and humoral acute phase-type reaction. In an orchestrated action, an inflammatory response is mounted in which activated micro- and astro-glia and their secreted inflammatory mediators and matrix constituents elicit the transformation of diffuse into neuritic plaques which, secondarily, may induce further tissue damage. Importantly, the AD brain is characterized by the microglial inability to clear the brain of the deposited material. The pathophysiological processes lead to functional and structural impairments of neuronal plasticity, a compromise of the neuronal network and, eventually, to cell death. Conspicuously, these alterations do not uniformly affect all brain regions but manifest with a temporal and topographical specificity vulnerable areas and nerve cell populations.

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KEY WORDS — Alzheimer’s disease; calcium; energy metabolism; oxidative stress; mitochondria; glucocorticoids; neuropeptide Y; DHEA; oestrogen; melatonin; somatostatin; nerve growth factors; insulin; $\beta$-amyloid; tau; immune system; signal transduction; apoptosis; vulnerability

AGEING AND ALZHEIMER’S DISEASE

In part I of this series it was posited that the age-related imbalance of neuropeptides and hormones puts the neurons under metabolic stress [Heininger, 1999]. Hence, it is outlined how this compromise of cellular homeostasis may lead to the production of amyloid $\beta$-protein (A$\beta$) and hyperphosphorylation of tau protein (P-tau). These processes occur already during normal ageing. Both A$\beta$ and senile plaques (SP) as well as P-tau and neurofibrillary tangles (NFT) are found in the normal aged brain in a spatial distribution suggesting their independent formation and potentially representing a preclinical stage of the disease [Beach et al., 1997; Braak and Braak, 1997; Troncoso et al., 1998; Price and Morris, 1999]. Nevertheless, since these changes are the hallmark of the pathomorphological diagnosis [Khachaturian, 1985; Ball et al., 1997] and, for the sake of the argument, they have been grouped as pathophysiological AD processes. However, it should be borne in mind that these processes are not AD-specific and that only by some additional quantitative and/or qualitative factors, identified as epidemiological risk factors (see part III of this series), these age-related processes turn into the pathomorphological hallmarks of AD. It is difficult to decide what is primary and what secondary in the sequence of events leading to AD. Looking back at the features already encountered during ageing will greatly assist in making these choices. These issues, however, will be largely postponed until part IV of this series and the present focus will be on the phenomenological description of the pathophysiological processes.

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β-AMYLOID PROTEIN FORMATION AND TAU PROTEIN HYPERPHOSPHORYLATION ARE SECONDARY EVENTS FOLLOWING NEURONAL METABOLIC STRESS

Amyloid precursor protein and β-amyloid

β-Amyloid is widely regarded as the centerpiece of the AD puzzle [Hardy and Higgins, 1992] due to the facts that (i) β-amyloid containing SP are one of the pathomorphological hallmarks of AD, (ii) amyloid precursor protein (APP) gene mutations are causally related to presenile AD, and (iii) β-amyloid appears to be neurotoxic in vitro and in vivo.

During the last 15 years, the biology of β-amyloid precursor protein (APP) and its metabolites has attracted much attention. It is beyond the scope of this series to cover all their multifaceted features. In the context of this paper, focus will be on the regulation of APP metabolism, particularly under conditions of derangements of cellular Ca2+ and energy homeostasis. The integration of Aβ and amyloid into the pathophysiological cascade will be attempted in part IV of this series. For further reading, several reviews are recommended which will also give the archival, pioneering literature [Mattson, 1994a, 1997; Robakis, 1994; Selkoe, 1994; Masliah, 1997].

Aβ, the main constituent of cerebral amyloid angiopathy [Glenner and Wong, 1984] and SP [Masters et al., 1985], is a proteolytic fragment of APP. The physiological role of APP is gradually emerging [reviewed by Masliah, 1997; Mattson, 1997; Panegyres, 1997]. It resembles a cell surface receptor, is expressed in a wide variety of cells and exists in different isoforms which are derived from alternatively spliced transcripts of a single gene on chromosome 21. With its single transmembranous domain and the 28 residues amino-terminal to it. There are other APP metabolites which comprises 11–15 amino acids of the trans-membrane region and the 28 residues amino-terminal to it. There are other APP metabolites which fulfills critical, partially isoform-specific functions in cell adhesion, neuronal differentiation, neurite outgrowth, neuronal activity, and synaptic plasticity and excitability. The growth-promoting, differentiation-regulating effects of APP are elicited in a joint, mutually-potentiating action with the nerve growth factor [Milward et al., 1992; Akar and Wallace, 1998] following upregulation of APP by different growth factors [Cosgaya et al., 1996] and neuronal differentiation [Yoshikawa et al., 1990; Fukuchi et al., 1992a]. Moreover, APP is upregulated in neurons, astrocytes and microglial cells in response to a multitude of cellular stressors. These include axonal injury [Gentleman et al., 1993; Blumbergs et al., 1995], loss of innervation [Wallace et al., 1993] and trophic factors [Araki and Wurtman, 1998], heat shock [Ciallella et al., 1994], inflammatory processes [Brugg et al., 1995], ischemia, hypoglycemia, brain trauma and excitotoxicity (see below), seizures [Panickar et al., 1998], oxidative stress [Frederikse et al., 1996], and ageing [Higgins et al., 1990; Adler et al., 1991; Nordstedt et al., 1991; van Gool et al., 1994]. Generally, APP confers protection against these stressors [Mattson et al., 1993a; Mucke et al., 1996; Masliah et al., 1997].

APP undergoes metabolism via two, mutually exclusive, pathways. In the secretory pathway, APP is cleaved by an as yet unidentified protease (dubbed α-secretase) just outside the transmembrane region to release the soluble ectodomain (sAPP). In the amyloidogenic pathway, a putative ‘β-secretase’ generates C-terminal fragments, further processed by a ‘γ-secretase’, to yield a hydrophobic peptide 39–43 residues long, Aβ, which comprises 11–15 amino acids of the transmembrane region and the 28 residues aminoterminal to it. There are other APP metabolites but focus will be on these biologically most relevant agents. APP is metabolized by complex, both intracellular and extracellular pathways which are not yet fully defined. The nascent forms undergo several posttranslational modifications upon transfer from the endoplasmic reticulum (ER) to the Golgi apparatus [Weidemann et al., 1989; Dugan et al., 1995]. APP is integrated into the cell membrane of perikarya and, after fast anterograde axonal transport [Koo et al., 1990], into synaptic membranes [Weidemann et al., 1989; Haass et al., 1992; Allinquant et al., 1994; Yamazaki et al., 1995]. There, APP is cleaved by α-secretase [Esch et al., 1990; Sisodia et al., 1990] to yield secreted sAPP. Lower amounts of secreted N-terminal β-secretase products have been detected also [Anderson et al., 1992; Tienari et al., 1997]. Secretion may require the full maturation of APP in the ER/Golgi. Mediated by sorting signals in its cytoplasmic tail [Chen et al., 1990; Lai et al., 1995], a fraction of full length APP and carboxyterminal APP fragments are re-internalized via coated pits and vesicles [Nordstedt et al., 1993; Yamazaki et al., 1996] and by retrograde axonal transport [Yamazaki et al., 1995] routed either to late endosomes/lysosomes [Haass et al., 1992; Refolo et al., 1995] or to the trans-Golgi network (TGN) [Stephens and Austen, 1996]. Endocytic recycling has been described also [Yamazaki et al., 1996; Yamazaki et al., 1995].

Koo et al., 1996]. Transcytotic trafficking [Simons et al., 1995; Yamazaki et al., 1995] may account for the dendritic APP pools [Allinquant et al., 1994; Simons et al., 1995]. Further intracellular processing is regulated by a network of intracellular trafficking between ER/TGN and endosomes/lysosomes. The relative importance of the individual cell organelles is modulated, depending on the constitutive or inducible secretory pathways, the Aβ variant produced, the intracellular accumulation or secretion of APP metabolites and the presence of APP mutations.

**How is the metabolism of APP regulated and what may be the signals which shift the APP metabolism from the non-amyloidogenic to the amyloidogenic pathway?**

The processing of APP is regulated by cell-cycle dependent phosphorylation/dephosphorylation balances [da Cruz e Silva et al., 1995; Desdouits et al., 1996; Gouras et al., 1998]. Phosphorylation cascades involving isoform-specific protein kinase C (PKC), PKA, phospholipase A2, phosphoinositol, mitogen-activated protein kinase and tyrosine phosphorylation steps are central to this regulation [reviewed by Mills and Reiner, 1999]. Stimulation of neurotransmitter receptors such as muscarinic, nicotinic, metabotropic glutamate and serotonergic receptors as well as growth factor-, interleukin- and neuropeptide-receptors and agents like arachidonic acid elicit the release of sAPP [reviewed by Mills and Reiner, 1999]. Intriguingly, these agents may cooperate by converging transduction pathways thus potentially potentiating sAPP secretion [Haring et al., 1998].

**Energetic stress modulates APP metabolism.** A consistent picture emerges based on a multitude of *in vivo* and *in vitro* findings: shortage of energy supply and Ca²⁺ overload not only induce an upregulation of APP but also route the metabolism of APP to the amyloidogenic pathway. Furthermore, increased APP expression [Fukuchi et al., 1992b] and certain mutations (discussed in part III of this series) direct the APP processing from the non-amyloidogenic to the amyloidogenic pathway. Ischemia, hypoglycemia and traumatic brain injury can induce upregulation of APP and its mRNA in animal models and culture systems [Abe et al., 1991; Hall et al., 1995; Murakami et al., 1998; Shi et al., 1998]. *In vivo*, excitotoxic stress induces APP expression in a boundary zone surrounding the necrotic core [Pangegyres, 1998] and upregulates APP in reactive astrocytes [Topper et al., 1995]. Thus, metabolic stressors elicit accumulation of cytotoxic Aβ-like fragments [Yokota et al., 1996] and possibly trigger deposition of β-amyloid in the human brain [Jendroska et al., 1995]. The accumulation of Aβ was reportedly more pronounced in aged than young rat brains [Popa-Wagner et al., 1998]. Chronic impairment of oxidative metabolism in the thiamine deficiency animal model leads to deposition of APP-like immunoreactivity in damaged brain regions [Calgingasan et al., 1995]. β-Amyloid can also be detected in human brain a couple of days after traumatic brain injury [Gentleman et al., 1993], a condition which has been shown to put neurons under metabolic stress [Xiong et al., 1997]. The potential mechanism has been elucidated recently. Fibroblasts from both AD patients and controls exhibited a reduced sAPP release under aglycemia. Furthermore, escalating energy compromise revealed an increased liability of AD fibroblasts and cultured neurons to reduced secretion of APP metabolites and amyloidogenic APP processing [Gasparini et al., 1997; Webster et al., 1998]. Inhibition of mitochondrial energy metabolism alters the processing of APP to generate a potentially amyloidogenic derivative [Gabusza et al., 1994; Mattson et al., 1998a]. Similarly, oxidative stress increased the expression of APP and production of Aβ [Frederikse et al., 1996]. Conversely, mitochondrial function may be compromised by Aβ [Ueda et al., 1994] so that in an evil cycle Aβ, generated following energy shortage, could sustain and exacerbate the mitochondrial failure.

**APP and Ca²⁺ mutually regulate their metabolism respectively homeostasis in a cellular stress feedback loop.** Evidence suggests that Ca²⁺ has a dual role in the regulation of APP metabolism with physiological Ca²⁺ levels augmenting the sAPP pathway while pathological Ca²⁺ levels favour Aβ generation. Neuronal activity with receptor-coupled activation of Ca²⁺ influx or intracellular Ca²⁺ release stimulates the secretion of sAPP in a PKC-dependent and PKC-independent manner [Löfler and Huber, 1993; Buxbaum et al., 1994; Savage et al., 1998], while Aβ production is inhibited [Buxbaum et al., 1993]. On the other hand, amyloidogenic proteolysis of APP was also enhanced by Ca²⁺ [Cheeler, 1995; Chong and Suh, 1996], by a Ca²⁺ ionophore in human platelets [Li et al., 1995] and in kidney cells transfected with APP cDNA [Querfurth and Selkoe, 1994]. The
ambiguous role of the Ca\(^{2+}\)/calmodulin system was demonstrated in a cell-free system by mediating opposing actions of PKC and calcineurin, a phosphatase, in the regulation of Aβ formation [Desdouits et al., 1996]. Thus, PKC activation at physiological Ca\(^{2+}\) levels may elicit APP metabolism by a Ca\(^{2+}\)-dependent z-secretase [Chen, 1997], induce sAPP secretion and inhibit Aβ production [Buxbaum et al., 1993; Hung et al., 1993], while the pathological Ca\(^{2+}\) level-induced inactivation of PKC [Darkin et al., 1996, 1997] may shift the APP metabolism to Aβ. Similarly, a defective PKC activity impairs the secretion of sAPP [Govoni et al., 1996] which may be reversed by PKC activation [Webster et al., 1998]. The cyclic AMP-dependent signaling pathway may both activate and inhibit the APP z-secretory pathway [Efthimiopoulos et al., 1996; Xu et al., 1996]. While PKC and cAMP/PKA have modulating effects on either APP catabolic pathway there may be a distinct regulation of sAPP and Aβ generation at the phosphatase level [da Cruz e Silva et al., 1995; Desdouits et al., 1996]. In the rat, it has been shown that such shifts in metabolic pathways must not necessarily involve different enzymes where the main APP-processing protease changed its proteolytic specificity towards amyloidogenic processing in the presence of Ca\(^{2+}\) [Kojima and Omori, 1992]. Thus, both metabolic pathways of APP are controlled by Ca\(^{2+}\) and possibly different Ca\(^{2+}\)-levels acting on the same or related proteases drive the metabolic shift. A similar situation is well established for the calpains, another Ca\(^{2+}\)-dependent protease family.

It has been demonstrated that both metabolic products of APP, sAPP and Aβ are operational in modulating cellular Ca\(^{2+}\) homeostasis and hence a role of APP in Ca\(^{2+}\) regulation has been proposed [reviewed by Mattson et al., 1993b; Mattson, 1994a; Fraser et al., 1997]. sAPP stabilizes G-protein dependently the neuronal Ca\(^{2+}\) homeostasis by K\(^{+}\)-channel-induced hyperpolarization and may thereby modulate synaptic activity [Morimoto et al., 1997]. The Ca\(^{2+}\)-regulating activity of sAPP was localized to a probably heparin-binding 22-amino-acid sequence at the C-terminus of the molecule [Furukawa et al., 1996]. sAPPβ, generated by β-secretase cleavage (together with Aβ) which carries only five residues of the Ca\(^{2+}\)-regulating sequence is approximately 100-fold less potent in attenuating Ca\(^{2+}\)-influx. Aβ, carrying the other 17 C-terminal residues displays opposite effects on neuronal Ca\(^{2+}\) homeostasis. Aβ induces Ca\(^{2+}\) entry either through activation of L-type and N-type Ca\(^{2+}\) channels [Ueda et al., 1997; Price et al., 1998], or through Aβ-assembled voltage-dependent ion channels [Ariese et al., 1993; Rhee et al., 1998]. Moreover, Aβ impairs Na\(^{+}\)/K\(^{+}\)-ATPase which may contribute to the derangement of Ca\(^{2+}\) homeostasis [Bores et al., 1998]. The disruptive effects of amyloidogenic APP processing on neuronal Ca\(^{2+}\) homeostasis may be twofold through Aβ-induced Ca\(^{2+}\)-influx and lack of sAPP-mediated inhibition of Ca\(^{2+}\)-influx. Thus, through Ca\(^{2+}\) regulation of APP expression and APP metabolism and control of Ca\(^{2+}\) by the metabolic products of APP, a feedback loop is established, which could explain a variety of physiological APP functions [Larner, 1995]. In a vicious circle Aβ promotes the expression of APP and decreases the release of sAPP so that, in an autocatalytic, ‘run-away’ process, further Aβ production may ensue [Takashima et al., 1995; Bahr et al., 1998]. Finally, Aβ avidly binds to APP and may thereby impair physiological functioning and proper processing of its precursor [Strittmatter et al., 1993].

The outlined features define a cellular stress role for APP. In analogy, heat shock proteins (hsp), another cellular stress system are upregulated by a variety of cellular stressors [Beckmann et al., 1992; Sharp et al., 1999], including increased cytosolic Ca\(^{2+}\) [Kiang et al., 1994]. Hsp stabilize intracellular Ca\(^{2+}\) [Katayama et al., 1994] and increase K\(^{+}\) currents [Negulyaev et al., 1996] and thereby protect mitochondrial respiration [Bornman et al., 1998] and neuronal viability against metabolic stressors [reviewed by Sharp et al., 1999]. The APP gene promoter shows sequence homology to the heat shock control element binding protein [Salbaum et al., 1988; Dewji and Do, 1996]. Thus, it is suggested that APP is expressed in response to cellular stress [Dewji and Do, 1996] and serves to regulate neuronal Ca\(^{2+}\) homeostasis. This function may be mediated by ligand-dependent binding of APP to the G protein G\(_i\) and activation of GTPase [Nishimoto et al., 1993; Okamoto et al., 1995] which regulate neuronal Ca\(^{2+}\) channels [Hescheler et al., 1987].

What are the neurobiologic effects of the APP metabolites?

sAPP and Aβ exhibit divergent effects on neuronal trophism and survival. Like hsp, both APP and sAPP are neurotrophic in a partially isoform-specific manner, thus completing the neuro-
and axonal sprouting. They enhance neuronal survival, neurite extension and axonal sprouting in vitro [Araki et al., 1991; Mattson et al., 1993a; Yamamoto et al., 1994] and increase synaptic density and memory performance in vivo [Roch et al., 1994; Meziane et al., 1998]. In the cortex of transgenic mice, human APP has a synaptotropic effect [Mucke et al., 1996]. APP 319–335, a 17-mer peptide, carries the neurotrophic effect of APP [Bowes et al., 1994; Yamamoto et al., 1994]. sAPP mediates cell–cell or cell–substrate adhesion [Milward et al., 1992]. sAPP was documented to protect against hypoxic, ischemic and excitotoxic neuronal injury [Mattson et al., 1993a; Bowes et al., 1994], attenuated glutamate-induced Ca\(^{2+}\) elevation and counteracted the inhibitory effect of glutamate on dendritic outgrowth [Mattson, 1994b; Moizumi et al., 1998]. The excitoprotectant activity of sAPP may be related to the direct suppression of NMDA currents [Furukawa and Mattson, 1998] and an increase of glutamate re-uptake by astrocytes due to an upregulation of glutamate transporter protein levels [Masliah et al., 1998]. Moreover, as discussed earlier, sAPP stabilizes the cellular Ca\(^{2+}\) homeostasis and glucose transport thereby protecting neurons against a variety of stressors.

The cellular actions of A\(\beta\) have been the subject of several reviews [Behl et al., 1994; Butterfield, 1997; Mattson, 1997; Mills and Reiner, 1999]. A wealth of data obtained with different doses and aggregation states suggest either a neurotoxic, neurotrophic or neuromodulating activity of A\(\beta\). Consistently in vitro but less so in vivo, \(\mu\)M concentrations of ‘aged’, fibrillar A\(\beta\) exhibit neurotoxic properties which are mediated by disruption of the Ca\(^{2+}\)-energy–redox homeostasis. Lower, physiological concentrations (pM to nM) of soluble A\(\beta\) exert both neurotoxic and neurotrophic effects. Together with findings obtained in familial AD and transgenic animal models (discussed in part III of this series), these results, although inconsistent, argue for a pathophysiological role of A\(\beta\)/amyloid in AD. In part IV of this series, the integration of these effects into a coherent concept may resolve many of these inconsistencies.

In AD brain, characteristically A\(\beta\) is deposited in amyloid plaques. The A\(\beta42\) variant is more fibrillogenic and amyloidigenic and functions as a seed that increases the kinetics of amyloid fibril formation [Jarrett and Lansbury, 1993]. Since the mutations linked to familiar AD and Down syndrome (discussed in part III of this series) increase the extracellular concentration and deposition of A\(\beta42\), it was suggested that A\(\beta42\) is the real culprit in AD [reviewed by Younkin, 1998]. This issue will be discussed in more detail in part IV of this series.

**Tau protein**

The biology and pathobiology of tau protein in AD has been reviewed repeatedly [Goedert, 1993; Holzer et al., 1994; Kosik and Greenberg, 1994; Trojanowski and Lee, 1995; Billingsley and Kincaid, 1997; Iqbal et al., 1998; Mandelkow and Mandelkow, 1998]. Physiologically, tau, is a phosphorylation- and isoform-dependent regulator of cellular cytoskeleton assembly and thus plays a role in neuronal development and differentiation, neuronal polarity, neurite outgrowth, neurite branching, synaptogenesis, intracellular trafficking and organelle transport, the activity of the cell-cycle machinery and apoptosis. The phosphorylation of tau is controlled antagonistically by the interaction of kinases and phosphatases which, particularly in the immature brain, dynamically mediate the delicately tuned phosphorylation of multiple epitopes and thereby cause rapid alterations in microtubule stability [reviewed by Billingsley and Kincaid, 1997; Lovestone and Reynolds, 1997]. These processes are subject to an array of signal transduction pathways elicited by hormones, growth factors, neurotransmitters and other agents such as APP metabolites, phospholipids and heparin in response to such diverse conditions as neuronal activity and synaptic input, growth, cellular metabolic stress and regeneration. As the final integrating agents Ca\(^{2+}\) and calmodulin regulate the phosphorylation of microtubule-associated proteins and microtubule assembly [Yamamoto et al., 1983]. In the differential regulation of tau isoforms and phosphorylation both temporal and spatial signals may be coded [Sadot et al., 1995; Mandell and Banker, 1996].

The principal component of neurofibrillary tangles (NFT), a major neuropathological lesion in AD brains are paired helical filaments (PHF). The main constituent of PHF is abnormally hyperphosphorylated protein tau (P-tau) which is believed to self-assemble into PHF. Tau induces bundling of tubulin into tightly packed parallel arrays and thick cables and, by stabilizing the microtubule assembly, plays an important role in cytoskeleton dynamics, axonal growth and transport and signal transduction [Gundersen and
As a consequence of hyperphosphorylation, tau is deficient to promote microtubule assembly with detrimental effects on these cellular processes. Phosphorylation at serine 262 appears to be particularly important for microtubule binding [Mandelkow et al., 1995]. Phosphorylation at Ser262 correlates with synaptic loss in AD brains while at Ser396/404 it does not [Callahan et al., 1998]. The upregulation of tau, as encountered in AD, may have similar effects on intracellular transport [Ebneth et al., 1998]. The microtubule disruption may also induce AD-like abnormalities of lysosomal functions [Shea, 1996].

Many candidate protein kinases have been suggested to participate in the regulation of tau phosphorylation and the generation of P-tau [reviewed by Lee, 1996; Mandelkow et al., 1996]. These include proline-directed kinases such as glycogen synthase kinase (GSK3), cdc2, cyclin dependent kinase (cdk)-5, mitogen-activated protein (MAP) kinase; non-serine-proline directed kinases like PKC, cyclic AMP-dependent protein kinase, Ca$^{2+}$/calmodulin-dependent protein kinase II, cusein kinase-1 and -2, protein kinase PKN; and serine directed kinases such as MAP/microtubule affinity regulating kinase (MARK) [Drewes et al., 1997]. Other kinases, belonging to the MAP kinase family, are activated by cellular stressors such as stress-activated protein (SAP) kinases [Carletti et al., 1995; Goedert et al., 1997], external-stimulus regulated kinases (ERKs) like PK40erk [Roder et al., 1993; Carletti et al., 1995]. Different kinases may act in concert and, respectively, up- or downregulate each other [Blanchard et al., 1994; Raghunandan and Ingram, 1995; Sengupta et al., 1997]. Protein phosphatases (PP)-1, PP-2A, PP-2B, and phosphotyrosyl-protein phosphatase (PTP) are the counterparts of kinases in the dynamic regulation of phosphorylation balances [reviewed by Trojanowski and Lee, 1995]. The control of phosphatases may be two-tiered by regulating the activity of kinases and by dephosphorylating tau [Tanaka et al., 1998].

An imbalance of kinase and phosphatase activities may be causally related to tau hyperphosphorylation in AD brains. Some kinases (e.g. GSK3, cdk5, casein kinase 1, Ca$^{2+}$/calmodulin-dependent protein kinase II) have been found elevated in AD brains preferably associated with NFTs [McKee et al., 1990; Yamaguchi et al., 1996; Vincent et al., 1998]. On the other hand, activity of phosphatases PP-1, PP-2A, and PTP towards abnormally phosphorylated tau was decreased, particularly in regions of prominent pathology correlating with NFT pathology. In tangle-bearing neurons, PP-2B staining was weaker than in unaffected neighbouring neurons and PP-2B activity correlated inversely with P-tau. In total, a similar to 30 per cent reduction of tau phosphatase activity has been reported in AD brains [reviewed by Iqbal et al., 1998].

Tau hyperphosphorylation precedes its polymerization into PHF/NFT and is already present in normal aged human and primate brain [Bancher et al., 1991; Delacourte et al., 1995; Gomez-Ramos and Moran, 1998]. P-tau may aggregate by hydrophobic interaction into tangles [Ruben et al., 1997], sequestering also normal tau into these filaments [Alonso et al., 1996]. Even normal tau is able to form PHF-like tangles when interacting with sulphated glycosaminoglycans like heparin [Goedert et al., 1996]. An oxidizing environment promotes the formation of intermolecular disulphide bridges and enhances the stabilization of the aggregates [Guttmann et al., 1995; Schweers et al., 1995]. Similarly, oxidative stress may augment the glycation of PHF, thus crosslinking and stabilizing the macromolecular PHF structure [Ledesma et al., 1994; Yan et al., 1994; Wang et al., 1996]. Following glycation, PHF and tau generate oxygen free radicals which may further sustain the pathophysiological vicious circles [Yan et al., 1995]. During maturation of PHF, the tangles are increasingly ubiquitinated [Mori et al., 1987; Iqbal et al., 1998] which further crosslinks the three-dimensional structure of the macromolecule.

What might be the processes leading to these pathophysiological tau alterations? In a variety of organ systems, subsequent to oxidative cell injury a disruption of the cytoskeletal organization and of the membrane–cytoskeleton interaction has been demonstrated [Bellomo and Mirabelli, 1992]. In the CNS, AD-type NFT occur in a variety of etiologically distinct neurologic disorders that show no amyloid deposition such as subacute sclerosing panencephalitis, lipofuscinosis, parkinsonism-dementia complex of Guam, encephalitic parkinsonism, ganglioglioma, Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, dementia pugilistica, and Hallervorden-Spatz disease [reviewed by Spillantini and Goedert, 1998]. Moreover, P-tau-like and PHF-like immunoreactivity accumulate under experimentally induced ischemia, heat shock and in human stroke [Johnson et al., 1993; Uchihara et al., 1995;
Bondereff et al., 1998]. Thus, NFT and tau aggregates can be seen as a non-specific response to noxious factors related to metabolic stress. Indeed, NFT but not SP are a marker of in vivo hypometabolism in AD [DeCarli et al., 1992]. Heat shock, osmotic or cold water stress, excitotoxic challenge, NGF deprivation, aluminum, Ca\(^{2+}\) influx and Ca\(^{2+}\) release can induce an AD-like tau hyperphosphorylation state [Johnson et al., 1993; Shea et al., 1996; Nuydens et al., 1997; Bondareff et al., 1998]. Likewise, glucose deprivation elicits NFT-like changes in cultured hippocampal neurons, mediated by neuronal Ca\(^{2+}\) influx [Cheng and Mattson, 1992]. These data are in contrast to findings that heat shock, ischemia, excitotoxicity, a rise of cytosolic Ca\(^{2+}\) levels and exposure to oxidative stress as well as compromise of mitochondrial function may also lead to dephosphorylation of tau [Norman and Johnson, 1994; Papasozomenos, 1996; Shackelford and Nelson, 1996; Adamec et al., 1997].

Differences of experimental conditions with regard to stressor dose and neuronal differentiation leading to differentially impaired cellular metabolic states, Ca\(^{2+}\) levels and oxidative stress may account for the discrepancies. Decline of ATP levels activates both a tau kinase and phosphatase [Bush et al., 1995]. Thus, Ca\(^{2+}\) elevations may affect tau phosphorylation biphasically, small increases leading to tau hyperphosphorylation and higher levels resulting in tau dephosphorylation and degradation [Hartigan and Johnson, 1998]. It is posited that tau hyperphosphorylation constitutes a cellular rescue programme to protect metabolically mildly stressed cells. Neurons expressing P-tau are more resistant to apoptosis [Lesort et al., 1997a] while tau dephosphorylation marks the onset of the apoptotic execution phase [Mills et al., 1998]. Intriguingly, P-tau colocalized with the outer mitochondrial membrane in heat shocked neurons, and porin, an outer mitochondrial membrane protein, was complexed to PHF-tau in AD brains [Bondareff et al., 1998]. These findings together with the role of microtubuli in the function of the mitochondrial permeability transition pore [Evtodienko et al., 1996; Linden and Karlsson, 1996] may indicate a hitherto unrecognized role of hyperphosphorylated tau in mitochondrial PT and apoptosis. Mechanistically, cellular metabolic stress resulting in cytosolic Ca\(^{2+}\) increase and partial depletion of ATP may activate both GSK-3\(\beta\) [Hartigan and Johnson, 1998] and MAP kinases, inactive under physiological conditions, which generate phosphoepitopes similar to those seen in AD [Roder et al., 1993; Shea et al., 1995; Goedert et al., 1997; Green et al., 1997; Luo et al., 1997]. Interestingly, the stress-activated kinases may be resistant to physiological inactivation by phosphatases [Roder et al., 1995]. Another missing link may be 4-hydroxynonenal, a product of lipid peroxidation, which increases tau phosphorylation and inhibits dephosphorylation of tau by binding directly to tau [Mattson et al., 1997].

Apart from features indicating cellular stress, developmental phenomena may play a pathophysiological role. Since foetal and postnatal tau are transiently phosphorylated in a manner similar to PHF-tau [Wolozin et al., 1988; Preuss and Mandelkow, 1998], it has been suggested that the abnormal phosphorylation of tau recapitulates developmental events [Wolozin et al., 1988; Goedert et al., 1993] which is in line with findings that neurons containing P-tau express cell cycle markers and appear to have re-entered the cell cycle [Nagy et al., 1997]. Remarkably, expression of mitotic tau phosphoepitopes precedes PHF formation [Vincent et al., 1998].

NFT predate SP formation, are a reliable marker for the progression of the disease [Braak and Braak, 1995, 1997] and correlate with a variety of disease markers such as oxidative metabolism, oxidative stress and neuronal death (see below). Thus, the generation of NFT is closely associated with the core processes of the disease. For instance, neurons bearing NFT, but not those containing P-tau alone, exhibited dramatically reduced levels of markers of synaptic plasticity [Callahan and Coleman, 1995]. Recently, the discovery that tau gene mutations cause frontal temporal dementia with parkinsonism linked to chromosome 17 [reviewed by Spillantini and Goedert, 1998] which resembles AD clinically, has also fuelled the concept that disruption of tau microtubule assembly is one of the key processes mediating neuronal dysfunction.

HORMONAL DYSREGULATION IN AD

A variety of neuroendocrine dysregulations and circadian alterations which evolve during ageing are aggravated in AD [Toutou and Haus, 1994; Magri et al., 1997]. In the AD brain, decreased neuromodulin Y (NPY)-like immunoreactivity [Chan-Palay et al., 1985; Beal et al., 1986], and NPY binding sites [Martel et al., 1990; Jacques et al., 1996] have been documented. The surviving cortical NPY-positive neurons showed somatic and

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dendritic abnormalities [Chan-Palay et al., 1985]. Moreover, CSF and plasma NPY levels are reduced [Alom et al., 1990; Koide et al., 1995], the reduction correlating with the duration of the disease [Minthon et al., 1996].

Conspicuously, an early age at menopause, i.e. the early lack of the protective action of oestrogen (E), is associated with a significant increased risk of AD [van Duijn et al., 1996; Sobow et al., 1999]. Likewise, surgically-induced menopause is a risk factor for an increased incidence and earlier age of onset [Hong-Goka and Chang, 1997; Nee and Lippa, 1999]. E-dependent signalling pathways may also be deficient in AD [Christie et al., 1990].

In comparison to elderly controls, plasma DHEAS levels were found to be reduced in AD patients [Yanase et al., 1996; Severgnini et al., 1998; Solerte et al., 1998] while DHEA levels did not differ significantly [Yanase et al., 1996] or were increased [Näsmann et al., 1995]. Moreover, the ratio DHEAS/GC was reduced in females but not males [Leblhuber et al., 1993]. In contrast, other studies could not detect different DHEAS levels in patient and control cohorts [Schneider et al., 1992; Ravaglia et al., 1998]. The picture was further confounded by recent studies finding that total 7α-hydroxy-DHEA levels, a DHEA metabolite, are increased [Attal-Khemis et al., 1998], that the adrenal stimulated release of DHEA is elevated in AD [Näsmann et al., 1995; Rasmusson et al., 1998], and that a lower DHEA level was associated with a better cognitive performance in AD patients [Miller et al., 1998]. Several factors may account for these conflicting data. The brain has its own de novo DHEA/DHEAS synthesis and homeostasis [Majewska, 1995; Robel and Baulieu, 1995] and rat and post mortem human brain DHEA levels were many times higher than plasma levels [Lanthier and Patwardhan, 1986; Lacroix et al., 1987]. In addition, adrenal DHEA production is increased upon stressful challenge concurrent with increased cortisol secretion [Bernton et al., 1995; Oberbeck et al., 1998] which may be another feature of DHEA's antiglucocorticoid actions [Regelson et al., 1994]. Thus, plasma DHEA levels may simply reflect the counterregulatory, with ageing declining capacity of the adrenal cortex [Hornsby, 1995], but may not allow conclusions about the pathogenetic relevance of DHEA in AD.

A significant melatonin (M) deficiency is common in AD compared to elderly controls [Maurizi, 1995]. It has been found decreased in the plasma [Touitou et al., 1984; Nair et al., 1986; Touitou and Haus, 1994; Uchida et al., 1996] and was particularly characterized by the lack of the nighttime peak and seasonal modulation. A considerable deficit has also been shown in the lumbar and ventricular CSF of AD patients [Skene et al., 1990; Tohgi et al., 1992; Liu et al., 1999].

Consistently, somatostatin (SS) has been shown to be decreased in AD brains and CSF [reviewed by Rubinow et al., 1995]. Likewise, SS receptors are diminished [Beal et al., 1985]. SS levels were (1) most reduced in brain areas most affected by AD such as temporal, frontal and parietal cortex; and (2) correlated with degree of cognitive impairment [Tamminga et al., 1987; Strittmatter et al., 1997], with the cholinergic deficit in temporal and frontal lobes [Dournaud et al., 1995], and with the degree of hypometabolism in PET scans [Tamminga et al., 1987]. Since GC regulate SS expression, the decrease of SS levels may be secondary to the GC increase [Wolkowitz et al., 1987; Papachristou et al., 1994]. The hypothalamic – pituitary – somatotropic (HPS) axis is hyporesponsive in AD and Down syndrome [Lesch et al., 1990; Arvat et al., 1996] resulting in a reduced stimulated release of growth hormone (GH). Remarkably, the blunted GH response correlated with the decrease of cerebral perfusion [Barquero et al., 1998], further strengthening the association of the HPS axis and brain vascular maintenance and remodelling [Sonntag et al., 1997]. As in ageing [see Heininger, 1999], peripheral insulin resistance may be routinely present in AD individuals. In comparison to normal controls, fasted plasma insulin levels were found higher or unchanged, but increased after an oral glucose tolerance test, while CSF insulin levels were reportedly lower or higher in AD patients [Bucht et al., 1983; Fujisawa et al., 1991; Craft et al., 1998]. Furthermore, after glucose administration, increase of blood glucose levels was higher and remained elevated for a prolonged period together with a marked improvement of memory performance [Craft et al., 1992]. Hyperinsulinemia is associated with recent onset AD in non-diabetic subjects [Kuusisto et al., 1997]. Since hyperinsulinemia may be a transient phenomenon in the course of AD [Craft et al., 1993], the discrepant data with regard to the control of glucose metabolism in AD patients may simply reflect different stages of the disease [Vanhanen and Soininen, 1998]. Brain insulin and c-peptide levels, insulin receptor and insulin-like growth factor (IGF) receptor densities in AD were found unchanged compared to elderly controls [Crews et al., 1992; De Keyser et al., 1994].

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In another study, insulin receptor densities were found increased in comparison to age-matched controls [Frölich et al., 1998]. IGF-1 immunoactivity was increased in a population of activated astroglia in the AD temporal cortex [Connor et al., 1997a]. In accordance with an earlier discussion [Heininger, 1999], expression of IGFs by astrocytes suggests a cellular stress response and the astroglial attempt to maintain neuronal homeostasis. Little is known about the state of the sensitivity of the insulin receptors. Indirect evidence suggests that the receptor is desensitized in AD [Henneberg and Hoyer, 1995]. Integrating the known disruption of IGF signalling pathways in ageing skeletal muscle [Renganathan et al., 1997], the dependence of the insulin and IGF receptor sensitivity and signal transduction on plasma membrane composition [Bruneu et al., 1987; Cremel et al., 1993; Nadin et al., 1994] and the severe disruption of AD plasma membrane composition (see part III of this series) into a coherent picture, a severe impairment of the receptor sensitivity and of secondary signalling processes can be assumed and, in fact, has been suggested at least in cortical brain areas [Frölich et al., 1998]. The IGF-binding protein (IGFBP)-2, the predominant CSF IGFBP and a major CSF protein, is produced by the choroid plexus [Ocran et al., 1990] which underlines the high importance of IGF for the brain function. IGFBP-2 and IGFBP-6 are increased in AD CSF [Tham et al., 1993]. IGFBP-6 seems to inhibit IGF action and has been implicated in growth inhibition [Babajko et al., 1997]. Since upregulation of the brain IGFBP-2 has been characterized as an injury-related response [Kleempt et al., 1992; Breece et al., 1996] this finding is in keeping with the metabolic stress state of AD and possibly indicates a functional deficiency of IGFs in the brain. An intriguing, but not yet investigated, possibility may be that the IGFBPs are phosphorylated which transforms them into inhibitors of the IGF action [Coverley and Baxter, 1997]. This may prevent the relay of serum IGFs into the brain and could interpret the reported increase of IGF-1 and IGF-2 in AD serum [Tham et al., 1993] as counterregulatory. Overall, the complex regulation of IGFs, their receptors and IGFBPs in the CNS is only fragmentarily understood and the interpretation of their dysregulation in AD is still speculative.

Differential brain region- and neurotrophin-specific alterations have been documented in AD brains. The NGF level is not decreased in AD serum and CSF, and both NGF and NGF mRNA levels have been reported either normal [Fahnestock et al., 1996; Hock et al., 1998] or increased [Scott et al., 1995; Narisawa-Saito et al., 1996] in the cerebral cortex and hippocampus of individuals with Alzheimer’s disease. NGF receptor expression is reportedly increased [Mufson and Kordower, 1992], but the high affinity trkA receptor expression and protein are reduced in AD cortex [Mufson et al., 1997; Hock et al., 1998]. In contrast, NGF levels, trkA expression and protein, and numbers of neurons expressing trkA, B and C are reduced in the AD basal forebrain [Boissiere et al., 1997; Mufson et al., 1997]. The link between the increased NGF levels in the cortex and the reduced levels in the basal forebrain was suggested to be a defective retrograde transport, the trk receptor deficit being the primary event [Mufson et al., 1995]. Likewise, it was concluded from the quantitatively different NGF receptor and ChAT reduction that the receptor deficit precedes the neuronal degeneration [Strada et al., 1992]. BDNF mRNA and protein is decreased in the hippocampus and temporal cortex in AD [Narisawa-Saito et al., 1996; Connor et al., 1997b], while NT-3 is lower in the motor cortex [Narisawa-Saito et al., 1996].

Thyroid hormone (TH) abnormalities occur regularly involving the whole cascade of regulation, production, transport and signal transduction. Subnormal levels of TH and/or elevated levels of TSH are common in demented and AD patients [Thomas et al., 1987; Faldt et al., 1996]. Most importantly, the transport of T4 into the brain appears to be insufficient since CSF transthyretin is reduced in AD [Serot et al., 1997; Merched et al., 1998], possibly since it acts as a chaperone and sequesters Aβ protein [Schwarzman and Goldgaber, 1996; Merched et al., 1998]. Thus, the odds ratio for the association between elevated TSH, as an indicator of a hypothyroid state, and dementia was significantly increased in a community-based study [Ganguli et al., 1996]. AD patients may show blunted responses to the TRH stimulation test [Thomas et al., 1987; Ceda et al., 1994] which, however, is not always different from aged controls [Stahelin et al., 1982]. AD patients may also exhibit reduced thyroid hormone receptor mRNA levels in the hippocampus [Sutherland et al., 1992] but elevated levels of TSH receptors in the temporal and frontal cortices [Labudova et al., 1999]. Finally, a high prevalence of antithyroid antibodies indicates an autoimmune thyroid disease in sporadic and familial AD [Ewins et al., 1991; Genovesi et al., 1999].
et al., 1996] and in individuals with DS contributing to the manifestation of AD [Percy et al., 1990].

Substance P-(SP)-like immunoreactivity is reduced in AD CSF [Cramer et al., 1985; Martinez et al., 1993] and SP-like immunoreactive neurons are depleted in AD cerebral cortex [Kowall et al., 1993; Ang and Shul, 1995]. SP receptor carrying neurons in the nucleus basalis degenerate selectively in AD brain [Kowall et al., 1993].

A link between a dysregulated hypothalamic–pituitary–adrenal (HPA) axis and both ageing and AD has been proposed [Deshmukh and Deshmukh, 1990; Orrell and O’Dwyer, 1995]. In early and late AD, the HPA axis shows multiple abnormalities of feedback regulation compared to healthy elderlies [Hatzinger et al., 1995; Näsman et al., 1996] resulting in a high incidence of escape from dexamethasone suppression [Gurevich et al., 1990; Hatzinger et al., 1995; Swanwick et al., 1998]. In AD patients compared with normal elderlies the total cortisol plasma concentration is increased and the circadian rhythmicity of cortisol levels is attenuated which is predominantly due to higher p.m. trough levels [Masugi et al., 1989; Touitou and Haus, 1994; Hartmann et al., 1997]. Moreover, progressive increase of baseline cortisol secretion and HPA axis hyperactivity correlated with the cognitive deterioration and hippocampal atrophy [de Leon et al., 1988; Gurevich et al., 1990; Weiner et al., 1997]. Likewise, cortisol levels in postmortem AD CSF were increased in comparison with elderly controls [Maeda et al., 1991; Swaab et al., 1994].

Due to the various endocrinological systems involved, only a full evaluation of the hormonal status and the related transport and signal transduction processes will give clues to the underlying deficits, identify individuals at risk and direct venues for treatment and prevention. Thereby, rather than the level of an individual hormone, the balance of neurotrophic/protective and neuro-aggressive hormones may characterize the risk profile of the individual patient. Only a full appreciation of this multidimensional network may prevent misleading conclusions about the pathological relevance of individual endocrinological axes.

The multifactoriality of the endocrinological dysregulation may have different consequences for discrete sets of neurons with their differential make-up of hormone receptors. The complexity of the situation is increased by the fact that the immune system, as an active player in the disease process, also is subject to the endocrinological changes and may even respond differentially to a given endocrinological pattern. A wealth of heterogeneity with regard to clinical, histopathological, and neurochemical features may result [e.g. Duguid et al., 1993; Mizutani et al., 1997]. The notion that the brain has only a limited battery of reactions to a variety of noxious stimuli is supported by the fact that there is still a common pathomorphological and -physiological basis (see part IV of this series).

THE PROGRESSIVE HORMONAL IMBALANCE FURTHER DETERIORATES THE HOMEOSTASIS OF THE CALCIUM–ENERGY–REDOX TRIANGLE

The endocrinological network provides the internal milieu which ensures optimal conditions for cellular welfare [Heininger, 1999]. In ageing, and even more pronounced in AD, the loss of the delicately tuned hormonal balance has detrimental consequences for the maintenance of the homeostasis of the interrelated, multifunctional messenger-effector web which is established by the vital Ca$^{2+}$– energy–redox triangle.

**Calcium homeostasis**

The paramount difficulties to assess the neuronal Ca$^{2+}$ homeostasis in ageing [Heininger, 1999] are even more true in AD due to the lack of an animal model. In a first approximation and in the light of the notion that AD is a systemic disease (see part IV of this series), peripheral cells from AD patients and in vitro models may give some clues. It should be kept in mind, however, that each tissue has its own distinct physiological and pathophysiological regulation of Ca$^{2+}$ homeostasis, with neurons and osteoblasts representing the extremes of a wide range of qualitative and quantitative differences [Fujita, 1986]. Hence, data obtained from peripheral cells only qualitatively may indicate abnormalities but may not allow conclusions about quantitative effects in brain cells. Extrapolating the evolving deficient control of Ca$^{2+}$ homeostasis in ageing [see Heininger, 1999], a dysregulation of Ca$^{2+}$ homeostasis in AD brain tissue cells with all its consequences for energy metabolism, oxidative stress, and sequelae for function and viability of neurons can be assumed.

The ‘calcium hypothesis of brain ageing and Alzheimer’s disease’ [Khachaturian, 1984] suggested a role of disturbed Ca$^{2+}$ homeostasis in the pathophysiology of AD (see vols 568 and 747 of
A reduced gastrointestinal absorption, decreased serum level of Ca\textsuperscript{2+} and bone mineral density indicate a general compromise of Ca\textsuperscript{2+} homeostasis [Ferrier et al., 1990; Landfield et al., 1991; Sato et al., 1998]. Lymphocytes display increased basal Ca\textsuperscript{2+} levels [Adunsky et al., 1991; Ibarreta et al., 1997], decreased stimulated uptake and release [Gibson et al., 1987; Grossmann et al., 1993; Bondy et al., 1996], decreased Ca\textsuperscript{2+} buffering capacities [Ibarreta et al., 1997] and deficient Ca\textsuperscript{2+}/calmodulin-mediated control of the activity of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger [Ibarreta et al., 1998]. Fibroblasts obtained from skin biopsies have advanced our understanding of AD-related alterations of Ca\textsuperscript{2+} homeostasis and signal transduction systems [reviewed by Gibson et al., 1996]. In sporadic and familial AD fibroblasts, Ca\textsuperscript{2+} homeostasis, e.g. total bound and free Ca\textsuperscript{2+} or inositol triphosphate-mediated Ca\textsuperscript{2+} mobilization is regulated abnormally and these alterations can already be detected in asymptomatic members of AD families manifesting the disease later [Etcheberriagay et al., 1998]. However, Ca\textsuperscript{2+} pools affected and direction of abnormality (either decrease or increase) may differ. AD fibroblasts show a decreased mitochondrial Ca\textsuperscript{2+} uptake, while under oxidative stress Ca\textsuperscript{2+} uptake is increased in comparison with age-matched controls [Kumar et al., 1994]. Cybrids obtained by fusion of mitochondria from AD platelets with a mitochondria-depleted neuroblastoma cell line displayed an increased basal cytosolic Ca\textsuperscript{2+} release and recovered more slowly from an elevation of cytosolic Ca\textsuperscript{2+} [Sheehan et al., 1997].

Direct and indirect evidence suggests a dysregulated Ca\textsuperscript{2+} homeostasis in AD brains. The ryano-dine and IP\textsubscript{3} receptors, both responsible for the stimulus-induced release of stored Ca\textsuperscript{2+}, are functionally severely compromised in regions with neurofibrillary and amyloid pathology resulting in a decreased binding of IP\textsubscript{3} to endoplasmic reticulum membranes [Haug et al., 1996; O’Neill et al., 1996; Kellifer et al., 1998]. Calmodulin, the integrator and coordinator of Ca\textsuperscript{2+} currents was found reduced in AD cortices by more than 60 per cent and additionally had a decreased ability to activate Ca\textsuperscript{2+}-dependent processes [McLachlan et al., 1987]. AD causes selective loss of calbindin-D28k from the cortex [McLachlan et al., 1987; Ferrer et al., 1993; Nishiyama et al., 1993] and from cholinergic neurons of the basal forebrain which precedes, is strongly related to, and considerably exceeds the cell loss [Ichimiya et al., 1989; Iacopino and Christakos, 1990; Lally et al., 1997]. In the hippocampus, the calbindin-D28k mRNA is reduced also [Maguire-Zeiss et al., 1995; Lally et al., 1997] rendering the affected cells less resistant to a variety of stressors [McMahon et al., 1998]. Another Ca\textsuperscript{2+}-binding protein, neurocalcin, was also found reduced in AD temporal cortex and may correlate with synaptic degeneration [Shimohama et al., 1996]. An elevated ratio of activated calpain I to its latent precursor (a Ca\textsuperscript{2+}-dependent cysteine protease and effector of Ca\textsuperscript{2+} signals which requires higher than normal Ca\textsuperscript{2+} levels for activation) is another feature indicating a compromised Ca\textsuperscript{2+} homeostasis in AD brains [Nixon et al., 1994]. The finding of an increased Ca\textsuperscript{2+} level in tangle-bearing neurons in autopic AD brain samples deserves mentioning [Garruto et al., 1984] but may be subject to and hence distorted by a variety of poorly controllable variables [see Heininger, 1999].

Finally, neurons and astrocytes from the trisomy 16 mouse, an animal model of Down’s syndrome and AD, displayed an increased level of basal and stimulated cytosolic and stored Ca\textsuperscript{2+} [Bambrick et al., 1997; Schuchmann et al., 1998; Cardenas et al., 1999]; neurons showed an increased expression of voltage-activated Ca\textsuperscript{2+} channels and a prolonged recovery of Ca\textsuperscript{2+} after an excitotoxic challenge with adverse consequences for mitochondrial energy homeostasis and viability [Galdzicki et al., 1998; Schuchmann et al., 1998; Cardenas et al., 1999].

**Energy homeostasis**

Qualitatively similar, in direct comparison with normal senescent controls, often quantitatively more pronounced deficits of energy metabolism have been reported in patients with AD.

Consistently, in vivo functional imaging techniques such as single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance spectroscopy (MRS), and dynamic MR imaging reveal alterations in cerebral blood flow, glucose and oxidative metabolism, and in high-energy phosphate metabolism characterizing the AD brain as in a hypometabolic state. Particularly, PET scans of the AD brains exhibit a severity-correlated reduction of glucose metabolism in the frontal and parieto-temporal region [DeCarli et al., 1992; Ibanez et al., 1998; Stein et al., 1998] which precedes and predicts cognitive decline [Haxby et al., 1986; Jagust et al., 1999].
1996] and in severe cases even affects the cerebellum [Ishii et al., 1997]. Close mapping of the affected regions disclosed a pattern of hypometabolism which paralleled the regional distribution of NFT routinely found in AD brains, e.g. highest in limbic areas of the temporal lobe and other prosocortical areas, while frontal lobe and unimodal association areas presented moderate changes and the motor/sensory cortices and occipital lobes were relatively spared [DeCarli et al., 1992; Stein et al., 1998]. Functional imaging suggests a diminished phosphorylation [Fukuyama et al., 1989] and transport of glucose [Friedland et al., 1989; Jagust et al., 1991] and a metabolic shift from glycolytic to oxidative metabolism [Fukuyama et al., 1994]. Thus, the global cerebral metabolic rate of oxygen was only slightly decreased due to an increased oxygen extraction fraction which, at least partially, compensated for the reduced blood flow [Tohgi et al., 1998]; the glucose extraction fraction and global cerebral metabolic rate of glucose, however, are significantly decreased, resulting in a significant elevation of the ratio of oxygen and glucose molar utilization in AD patients [Kuwabara et al., 1996; Ogawa et al., 1996]. These alterations may indicate the uncoupling of the mitochondrial tricarboxylic acid cycle (TCA) from the respiratory chain as discussed for conditions of mitochondrial Ca2+ overload [Heininger, 1999]. Such an uncoupling may also affect the pattern of TCA substrates, which in fact has been demonstrated in AD CSF [Redjems-Bennani et al., 1998]. Of note, in vivo N-acetyl aspartic acid levels, a marker of neuronal viability correlated with the reduction of glucose metabolism and hippocampal atrophy [Schuff et al., 1997; Andersen et al., 1998].

Notably, when cognitively challenged, the AD brain is unable to activate respective brain regions [Corkin et al., 1998; Rombouts et al., 1998], to direct blood flow to the active brain regions [Matteis et al., 1998] and to comply with the increased energetic demand [Kessler et al., 1990; Hock et al., 1997]. Hence, glucose metabolism during stimulation is a more sensitive marker of functional metabolic failure than at rest [Pietrini et al., 1999]. This is compatible with a deficit of mitochondrial energy homeostasis, since mitochondria with deficient respiratory chain are unable to increase the glucose-phosphorylating enzyme activity above the basal state [Gerbitz et al., 1996]. With 31P-MRS, a severity-dependent reduction in measures of high-energy phosphates was detected and suggested that the AD brain is under energetic stress [Pettegrew et al., 1994; Mecheri et al., 1997].

Biochemically, a series of in vivo, ex vivo and postmortem studies clearly indicate an impaired mitochondrial function. Mitochondrial numerical density decreases exceed the ageing-related extent [Bertoni-Freddari et al., 1997]. While in ageing the lower density may be compensated by an increase of mitochondrial volume, in AD this compensatory response is less efficient [Bertoni-Freddari et al., 1997]. Glucose metabolism and ATP production is deficient [reviewed by Blass, 1993; Hoyer, 1993; Meier-Ruge et al., 1994] which correlates with the clinical severity of the disease [Hoyer et al., 1991]. The compromise of glucose and energy metabolism in AD exceeds the ageing-related decrease [Swerdlow et al., 1993] and includes reduced glucose transporter concentrations [Kalaria and Harik, 1989; Simpson et al., 1994; Harr et al., 1995], downregulation of glucose transporter gene expression [Jacobs et al., 1997] and decreased activities of pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KGDH) [reviewed by Kish, 1997; Gibson et al., 1998], two rate-limiting key enzymes of mitochondrial oxidation which stand under Ca2+ control. Moreover, a deficiency of the electron transport chain and particularly of cytochrome oxidase and its mRNA was reported [reviewed by Kish, 1997; Gibson et al., 1998] leading to a decreased content of adenine nucleotides in biopsy specimen [Sims et al., 1983]. The decrease of cytochrome oxidase mRNA precedes the formation of NFT and further decreases as NFT formation progresses [Hatanpää et al., 1996]. Surprisingly, neurons near plaques did not present cytochrome oxidase mRNA reductions suggesting that plaques may not contribute to decreased energy metabolism in AD [Hatanpää et al., 1998]. Transfer of mitochondrial DNA from AD platelets into neuronal cells which have been depleted of their mtDNA yields so-called cybrids. These exhibit a cytochrome oxidase defect and oxidative stress which, by opening the permeability transition pore, lowers the mitochondrial membrane potential and elicits features of apoptosis [Sheehan et al., 1997; Cassarino et al., 1998].

The pathogenetic relevance of these findings has been disputed and the hypometabolism was interpreted as secondary [reviewed by Chandrasekaran et al., 1996]. Undoubtedly, a primary loss of neuronal connections and activity as seen in AD will be followed by a secondary decrease of energy production — according to the stimulus—
response–metabolism coupling [see Heininger, 1999]. However, a variety of features [reviewed by Blass, 1993] indicate that the metabolic alterations in AD cannot be attributed and thus appear not to be secondary to the impairment of neuronal activity. (1) Even after correction for brain atrophy glucose metabolic values remain reduced [Ibanez et al., 1998]. (2) In early AD, the pronounced reduction of glucose utilization compared with the discrete decrease of oxygen consumption points to a disturbance in the control of glucose metabolism as the cause rather than the consequence of neuronal loss in AD [Hoyer et al., 1991]. (3) In contrast to AD, these changes could not be found in vascular dementia [Kuwabara et al., 1996]. (4) The dissociation between decreased metabolic rate of oxygen and increased oxygen extraction fraction in early AD cannot be explained by secondary adaptive changes but point at a primary misery perfusion syndrome [Nagata et al., 1997]. (5) The suggested physiological adaptations of supply to demand are not compatible with the elevated oxygen/glucose molar utilization ratio. (6) Asymptomatic or only mildly amnesic individuals at risk of familial or sporadic AD (which became manifest later) exhibit a parietotemporal and mediotemporal hypometabolism in PET [Reiman et al., 1996; Minoshima et al., 1997] and anecdotal evidence from an asymptomatic individual indicates a very early derangement of high-energy phosphate metabolism years before the clinical manifestation of AD [Pettegrew et al., 1995]. (7) The reduction of temporal blood flow, regional perfusion and glucose metabolism precede and predict the structural brain changes and clinical manifestation [Johnson et al., 1998; Julin et al., 1998].

As possible genetic origin for the metabolic impairment, an increased incidence of mtDNA mutations was suggested [Beal, 1995; see part III of this series]. Compared to age-matched controls, oxidative damage to mtDNA increases. A high level of mtDNA deletions [Blanchard et al., 1993; Corral-Debrinski et al., 1994] and a threefold increase of oxidized mtDNA nucleosides [Mecocci et al., 1997] have been documented. The increased oxidative stress (see below) was suggested as causally related to both the acquired mtDNA mutations and a decreased mitochondrial membrane fluidity [Mecocci et al., 1997]. An impairment of mtDNA amplification in inferior parietal cortex specimen correlated with the cognitive impairment [Brown, A. M. et al., 1998]. Based on the genetic findings a mitochondrial bottleneck hypothesis of AD has been formulated [Davis et al., 1995].

Antioxidant/oxidant homeostasis
A wealth of evidence implicates oxidative stress-related mechanisms in the pathophysiology of AD [reviewed by Harman, 1993; Ceballos-Picot, 1997; Markesbery, 1997]. Ca$^{2+}$-mediated uncoupling of TCA cycle and electron transfer transforms mitochondria into a virtually inexhaustible source of reactive oxygen species in aerobic conditions [Dykens, 1994; Benzi and Moretti, 1995]. Other processes generating oxygen radicals may include metabolic pathways of arachidonic acid, NO, Aβ and microglial oxidative burst. Ageing per se is associated and may be dependent on a variety of oxidative reactions of nucleic acids, proteins and lipids [Heininger, 1999]. The brain is particularly susceptible to oxidative damage due to its high oxygen consumption rate, relatively low level of antioxidant enzymes and high lipid content [Coyle and Puttfarcken, 1993].

Oxidative stress in AD brains was documented by assessing oxidative reaction products, associated processes and changes in antioxidant enzymes. Various oxidative stress-related nonenzymatic reaction products have been found increased in AD brains. Brain regional AD histopathology corresponded with biomarkers of protein oxidation [Smith et al., 1991; Lyras et al., 1997]. Carbonyls and nitrotyrosine, products of oxidative damage, were increased in CSF and brain and particularly were found associated with NFT [Smith et al., 1991, 1998; Lyras et al., 1997]. Lipid peroxidation was selectively increased in the inferior and medial temporal cortex and hippocampus [Palmer and Burns, 1994; Lovell et al., 1995]. A lipid peroxidation product, 4-hydroxy-2-nonenal (HNE), is a potent neurotoxin, crosslinker of cytoskeletal proteins [Montine et al., 1996] and inhibitor of key enzymes of glycolysis and TCA cycle, PDH and KGDH [Humphries and Szewda, 1998]. It was detected elevated in AD ventricular fluid [Lovell et al., 1997] and brains [Markesbery and Lovell, 1998], again preferentially in NFT while Aβ deposits were unlabelled [Sayre et al., 1997]. Of note, the increase of HNE adducts seemed to be at least in part associated with apoE4 inheritance [Montine et al., 1997]. A marker of oxidative damage of proteins, acrolein, also localized with NFT, but not with SP core [Calingasan et al., 1999]. Homogenized autopsy samples of AD cortex
show increased susceptibility to lipid peroxidation and oxygen radical formation in vitro [Subbarao et al., 1990; McIntosh et al., 1997].

Reactive oxygen species (ROS) can induce heat shock protein (hsp) synthesis in neurons and hence hsp expression may serve as marker of oxidative stress [Omar and Pappolla, 1993]. In fact, increased synthesis and accumulation of hsp72, 70 and 27 was observed in AD brains [Hamos et al., 1991].

Another indicator of oxidative stress, glycation of proteins, is a slow process which therefore affects primarily proteins with a slow turnover. Advanced glycation end products (AGE) result from post-translational condensation of proteins with reducing sugars in the Maillard reaction [Monnier and Cerami, 1981]. Glycation and oxidation mutually affect each other in a synergistic and additive way [Yan et al., 1995; Traverso et al., 1997]. AGE were found increased in NFT and SP of AD brains [Smith et al., 1994a; Vitek et al., 1994; Dickson et al., 1996], AGE reportedly are strongly associated with PHF, involving the tubulin-binding domain of tau [Ledesma et al., 1994; Yan et al., 1994] and with apoE [Dickson et al., 1996]. AGE were also increased in lipofuscin pigments of AD brains [Horie et al., 1997]. Cumulative evidence, however, suggests that AGE labelling of SP is not associated with Aβ [Kimura et al., 1995; Tabaton et al., 1997], but the neurofibrillar structures [Horie et al., 1997] and coronas of SP and accompanying microglial reaction [Kimura et al., 1995; Dickson et al., 1996; Takeda et al., 1998].

The evidence for increased oxidative damage to mtDNA was reviewed above. Likewise, nuclear DNA oxidation is enhanced in AD cortices [Gabbita et al., 1998; Lovell et al., 1999]. The repair product, however, was decreased in AD CSF [Lovell et al., 1999]. This occurs although DNA repair enzymes may be upregulated in AD brains [Hermon et al., 1998], suggesting a high intensity of the oxidative challenge and the functional inadequacy of repair mechanisms.

Rather than the absolute levels of antioxidant enzymes, the balance of the activity of oxidants such as active oxygen species and transition metals and of antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and vitamins E, C, and A, may determine the susceptibility of tissues to oxidative damage [McCord, 1993] and failure of energy metabolism [Davey et al., 1998]. Overall, antioxidant enzymes in AD brains have not shown a consistent pattern. Increased, reduced or unchanged differential enzyme-specific and cell type-specific localizations and activities of antioxidants such as SOD, CAT, and GPX and glutathione were found in autopic AD brain samples [reviewed by Pappolla et al., 1996; Ceballos-Picot, 1997]. In contrast, SOD activity was reduced in the CSF of AD patients [Bracco et al., 1991; DeDeyn et al., 1998]. It is suggested that the heterogeneity of results is related to various factors: (1) length of agonal state shifting the apoptosis/necrosis equilibrium which affects the antioxidant balance (see below); (2) postmortem delay; (3) sampling from different brain areas exhibiting varying antioxidant balances; (4) antioxidant enzymes can be glycated, followed by fragmentation [Okawara et al., 1992; Takata et al., 1996]. The glycation may affect activity and antigenicity differentially and inconsistently [Adachi et al., 1991; Yan and Harding, 1997] and hence histochemical detection may not reflect enzymatic activity levels. In another run-away process the glycation of antioxidant enzymes may further enhance oxidative stress [Fuji et al., 1996]. On the other hand, consistent findings have been reported for heme oxygenase-1, a stress response protein which converts heme to the antioxidant progenitors biliverdin/bilirubin which protect neurons against oxidative stress [Dore et al., 1999]. Heme oxygenase-1 and its mRNA were found elevated in association with the neurofibrillary pathology of the frontal, temporal and occipital lobes but not the cerebellum [Smith et al., 1994b; Schipper et al., 1995]. Nitric oxide synthase suggesting potentially neurotoxic NO production was expressed in NFT-bearing neurons [Vodovotz et al., 1996] and in reactive astrocytes in areas with high densities of SP [Wallace et al., 1997]. In summary, the topographical distribution of markers of oxidative stress indicates an association with NFT and the elements of the cellular response to SP (see below).

In AD blood, alterations of antioxidant balance indicate also a systemic oxidative stress. AD red blood cell SOD and GPX levels were increased [reviewed by Ceballos-Picot, 1997], as were blood hydroxyl radical and lipid peroxidation levels [Smith et al., 1998]. In addition, plasma and CSF oxidizability were increased and levels of antioxidants and antioxidant capacity reduced [Jeanel et al., 1989]. Remarkably, the erythrocyte SOD levels were also increased in first degree relatives of AD patients [Serra et al., 1994]. A marker of oxidative stress in DNA was elevated in AD lymphocytes [Mecocci et al., 1998]. It should be
kept in mind that the potent cellular antioxidants melatonin (as scavenger roughly five times more effective than glutathione; Reiter et al., 1996), oestrogen and DHEA are reduced in AD brain, CSF, and plasma (see above). Features of alterations of trace metals and antioxidant vitamins indicating a propensity for increased oxidative reactions and modulation of counterregulation will be discussed in part III of this series. Finally polyunsaturated fatty acids, a target of lipid oxidation was found decreased in high density lipoproteins, CSF, ventricular fluid and brain tissue of AD patients [Montine et al., 1997; Prasad et al., 1998]. Features indicating increased oxidative stress are also present in a variety of peripheral cells (discussed in part IV of this series).

HORMONAL DYSREGULATION AND APP/TAU METABOLISM

Several lines of evidence suggest that hormonal dysregulations directly, or by mediation of the deranged homeostatic triangle, underlie or at least contribute to both APP mismetabolism and neurofibrillar pathology.

Neuropeptides and hormones can interfere with APP metabolism and Aβ effects. Thus, neurohormones with Ca²⁺ antagonistic/ROS scavenging properties inhibit Aβ and promote sAPP formation. Melatonin affects the processing of APP in cell lines [Song and Lahiri, 1997] and protects neurons against Aβ toxicity [Papolla et al., 1997; Daniels et al., 1998]. E attenuates APP overexpression in a focal ischaemia animal model [Shi et al., 1998]. Both E and DHEA direct APP metabolism into the secretary pathway [Jaffe et al., 1994; Danenberg et al., 1996; Xu et al., 1998] and inhibit the Aβ pathway [Chang et al., 1997; Liu et al., 1998]. E and IGF-1 protect neurons against Aβ toxicity [Green et al., 1996; Dore et al., 1997]. Thyroid hormones repress APP expression and regulate APP gene splicing and hence, expression of isoforms and may modulate APP metabolism [Belandia et al., 1998; Latasa et al., 1998]. In contrast, GC enhance the production of Aβ [Liu et al., 1998] while a GC receptor antagonist increases the secretion of sAPP [Lam and Reiner, 1996].

The hormonal imbalances may also affect the phosphorylation/dephosphorylation balance of tau. Testosterone was shown to prevent the heat shock-induced hyperphosphorylation in male rats [Papasozomenos, 1996]. Likewise, insulin and IGF-1 regulate tau phosphorylation in cultured human neurons by downregulating the GSK-3 activity [Hong and Lee, 1997]. M is able to reverse the action of the protein phosphatase inhibitor okadaic acid [Cozzi and Rollag, 1992] which induces tau hyperphosphorylation and PHF-like pathology. Finally, thyroid hormones regulate microtubule assembly, tau expression and splicing [Fellous et al., 1979; Nunez et al., 1991]. It is speculated that the lack of androgens, M, TH and IGF-1, in ageing and AD is involved in modulating microtubule gene expression, and the kinase/phosphatase balance, contributing to tau hyperphosphorylation and microtubular pathology. Finally, the DHEA/DHEAS balance may affect the polarity of neurons [Compagnone and Mellon, 1998] as a function of the microtubule composition, and the loss of polarity in AD may be associated with an altered DHEA/DHEAS balance.

THE IMMUNE SYSTEM IS DYSREGULATED AND CONtributes TO THE PATHOPHYSIOLOGY OF AD

Alzheimer’s disease and peripheral immune system

In AD, the immune system largely displays features which, relative to ageing, are similar although more pronounced but also some which are qualitatively different [Antonacci et al., 1990]. Evidence indicates a decreased cellular and increased humoral immunity [Ikeda et al., 1991]. AD lymphocytes appear to be relatively anergic [Trieb et al., 1996; Eckert et al., 1997]. T-cells exhibit a decreased proliferative response upon stimulation [Singh et al., 1986; Nijhuis et al., 1991]. Binding of IFN-γ is reduced while binding of interleukin (IL)-6 is increased [Bongioanni et al., 1998]. The activity of natural killer (NK) cells was reported to be reduced [Kraus, 1983], but compared to controls was found increased after cytokine stimulation [Solerte et al., 1998]. The functional deficits may be related to the compromise of cellular Ca²⁺ homeostasis and defects of signal transduction processes. IgG and IgA are increased and the increase correlated with the cognitive impairment [Eisdorfer and Cohen, 1980; Leblhuber et al., 1998]. The loss of specificity and of self–nonself discrimination of the humoral immune system is highlighted by the presence of a variety of autoantibodies in serum and CSF [reviewed by Percy, 1993] and of circulating immune complexes [Heinen et al., 1993]. The serum and CSF in AD patients indicate an acute phase reaction. AD blood macrophages are chronically
activated [Hartwig et al., 1998], mononuclear cells show an elevated interleukin secretion compared with elderly controls [Huberman et al., 1995]. Blood IL-2 is found increased only in later stages of the disease while IL-1 and IL-6 levels are increased already in early stages, and correlate with the severity of dementia in AD and Down syndrome [Huberman et al., 1995; Alvarez et al., 1996; Kalman et al., 1997; Singh and Guthikonda, 1997]. Acute phase proteins α1-antichymotrypsin, ceruloplasmin, complement components C3, C4, properdin factor B, C-reactive protein, and tumor necrosis factor (TNF) were found increased in serum [Giometto et al., 1988; Matsubara et al., 1990; Furby et al., 1991] as well as soluble TNF- and IL-2 receptors [Leblhuber et al., 1998]. IL-1β, IL-6 and ACT are also elevated in CSF [Matsubara et al., 1990; Blum-Degen et al., 1995].

The decay of the hormones/neuropeptides DHEA, melatonin, neuropeptide Y, IGF, and somatostatin on the one hand and the dysregulation of the HPA axis on the other hand affect the immune system in a specific way (see Heininger, 1999 and part V of this series). The imbalance between the hormones may be sufficient to drive the immune system into the acute phase type reaction.

Alzheimer’s disease and cerebral immune response

Affected by the same decay of hormonal homeostasis as the neural system, the immune system in AD patients exhibits an inflammatory reaction to parenchymal deposits compatible with an acute phase response [Bauer et al., 1991; Eikelenboom et al., 1991]. In contrast to occasional resting microglia primarily in the white matter of nondemented elderlies, AD brains contain clusters of activated microglia in white and grey matter [Styren et al., 1990; Eikelenboom et al., 1993]. While diffuse Aβ deposits in elderly controls contain only quiescent microglia, activated microglia were found associated with the neuritic evolution of plaques [Eikelenboom et al., 1993; Mann, 1993; Sheng et al., 1995, 1996; Wisniewski et al., 1996]. Both activated microglia and astrocyes have been found correlating with progressive stages of neurofibrillar tangle formation and degeneration of tangle-bearing neurons [Pike et al., 1995; Sheng et al., 1997]. Activation of microglia seems to increase along the stages of plaque maturation, is first detectable at the transition between diffuse and primitive plaques and progresses to clusters of activated microglia in classic plaques [Susaki et al., 1997] paralleled by the neuritic transformation of SPs [Su et al., 1998]. Compelling histological evidence indicates that microglial and perivascular cells are directly engaged in the production of fibrillar amyloid [reviewed by Wisniewski et al., 1996; Dickson, 1997; Wisniewski, 1997]. Amyloid fibrils appear first in the altered cytoplasmic membrane and deep infoldings of microglial plasma membranes [Frackowiak et al., 1992]. The inability to detect APP mRNA in microglial cells makes them an unlikely source of Aβ but emphasizes their role in Aβ processing [Scott et al., 1993; el Hachimi and Foncin, 1994]. In addition, matrix proteoglycans which are secreted by activated microglia and astrocytes [Miller et al., 1997; Dow and Wang, 1998] are present in the SP, bind to Aβ, accelerate Aβ fibril formation, and maintain Aβ fibril stability [Snow et al., 1994a, b; Castillo et al., 1997]. Thus, cellular and matrix constituents which also include trace metals (discussed in part III of this series) may jointly effect the processing of deposited aggregated Aβ into fibrillar material. Similarly in transgenic mice and a rat Aβ infusion model, SP formation was associated with a profound microglial response [Frautschy et al., 1998a; Netland et al., 1998]. In vitro, rodent macrophages or microglia exposed to fibrillar Aβ show an enhanced oxidative burst [McDonald et al., 1997; Vitek et al., 1997] and phagocytic and neurocystopathic activity [London et al., 1996; Giulian et al., 1996; Kopec and Carroll, 1998]. Likewise, Aβ, in an aggregation-dependent manner, elicited astrocytic activation [Pike et al., 1994]. In human macrophages, however, Aβ failed to induce an oxidative burst and even inhibited the apo E-stimulated NO release [Vitek et al., 1997].

In the AD brain, typically a variety of inflammation-related molecules are either uniquely present or significantly elevated in comparison to samples from nondemented elderlies [Rogers et al., 1996]. Characteristically these acute phase reactants also differentiate amorphous plaques in the AD cortex from those in the cerebellum [Rozenmuller et al., 1990]. It was suggested that these agents establish a self-propagating ‘cytokine cycle’ which drives the progression of the disease and plaque formation [Griffin et al., 1998]. The mediators, most of them secreted by activated microglial and astroglial cells, orchestrate a cellular and humoral inflammatory response of the unspoken acute phase response type, directed against the disease-associated parenchymal deposits [reviewed by Rogers et al., 1996;
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Griffin et al., 1998; McGeer and McGeer, 1998). The agents include the full range of the classical complement cascade, cytokines such as IL-1x, and β, IL-6, TNF, and acute phase reactants like α-2-macroglobulin and α-1-antichymotrypsin. Proteins of the complement cascade can be produced in the normal brain but are elevated several fold in AD brains [McGeer et al., 1992; Yasojima et al., 1999]. Both, diffuse and neuritic plaques contain proteins of the complement cascade [Eikelenboom and Stam, 1982; Eikelenboom et al., 1993]. Notably, the membrane attack complex C5b-9 is abundant in AD brains, associated with neurofibrillary patholgy but was hardly detectable in nondemented elderslies [Webster et al., 1997a]. Concomitantly, the membrane inhibitor of reactive lysis (CD59) and complement inhibitors such as clusterin, vitronectin and protectin are upregulated in AD brains and may protect cells against complement lysis [McGeer et al., 1991, 1992]. Transgenic mouse models and Aβ infusion models of AD emphasized the relevance of an active complement response for a microglial SP-related activation [Frautschy et al., 1998b; Wright et al., 1998]. IL-1 and IL-6, which play a critical role in the orchestration of the acute phase response, are both elevated in AD brains and may mediate the autocrine propagation of an inflammatory cycle characterized by the proliferation of microglial cells and upregulation of IL-1, IL-6 and TNF [reviewed by Mrak et al., 1995]. Furthermore, IL-1 may contribute to the downregulation of cerebral neuropehphin expression [Lapchak et al., 1993], impairment of long-term potentiation [Murray and Lynch, 1998] and ageing-related loss of Ca2+ homeostasis [Campbell et al., 1998]. Expression of glial IL-1x and IL-6 is associated with the development of neuritic plaques [Sheng et al., 1995; Hüb et al., 1996]. On the other hand, IL-1 and IL-6 are able to induce APP in neurons, establishing another dynamic vicious cycle [Goldgaber et al., 1989; Griffin et al., 1998]. Overall, the importance of inflammatory processes in the clinical manifestation of AD is suggested by the epidemiological evidence of a protective action of non-steroidal antiinflammatory drugs [Breiten et al., 1994] which was substantiated in a small prospective trial [Rogers et al., 1993].

What renders the immune system of AD patients reactive to the deposits while nondemented elderslies do not mount an immune response? In vitro findings suggest that glial IL-1β and IL-6 release, TNF-α production and respiratory burst are activated by Aβ and the APP carboxy-terminal, particularly in the aggregated state [Meda et al., 1995; McDonald et al., 1997] resulting in an increased neurocytopathic effect of monocytes [London et al., 1996; Weldon et al., 1998]. Particularly, complement is activated by β-amyloid aggregation state-dependently via both the classical and alternative pathways [Rogers et al., 1996; Webster et al., 1997b], possibly affecting complement dependent lysis in the proximity of fibrillar but not diffuse Aβ deposits and forming an inflammatory nidus for further recruitment of microglia. Notably C5a, a chemoattractant for microglial cells cooperates with Aβ to induce IL-1 and IL-6 release from microglial cells [O’Barr and Cooper, 1998]. However, as exemplified in the non-demented, high amyloid burden carriers in which immune activation phenomena are virtually absent, amyloid deposits alone may fail to trigger a substantial immune response. AD is a systemic disease (this will be outlined in part IV of the series) and as the disease progresses concomitant changes of the immune system (see above) increase the propensity of the innate immune system for primitive, acute phase response-like reactions. The brain itself may play a key role in the promotion of immune senescence. Lesion of the cholinergic basal forebrain elicited an anergic ‘senescent’ state of the cellular and humoral immune system in laboratory animals [Popovic et al., 1997]. The noradrenergic system may equally be involved. The disease-associated degeneration of the cholinergic and noradrenergic system (see below) thus may also drive the progression of the inflammatory component and may finally be instrumental in the full-blown pathomorphological manifestation of the disease. In analogy to the ageing brain [Li et al., 1998], the initial trigger to set in motion the inflammatory cascade may come from the advanced glycation endproduct (AGE) system. Interaction of Aβ with receptors for AGE elicits in neurons (and possibly in astrocytes) the expression and secretion of macrophage-stimulating factor (M-CSF), an activator of macrophages [Du Yan et al., 1997]. Presumably, the inflammatory cascade can also be triggered by fibrillar Aβ, which is a chemoattractant for macrophages [Davis et al., 1992].

An issue which has received relatively little attention so far is the reason why microglia, the resident macrophages (‘big eaters’), and astroglial cells although they have been shown to contain amyloid material [Frackowiak et al., 1992; el Hachimi and Foncin, 1994; Yamaguchi et al., 1998] may be unable to clear the brain from the

amyloid deposits. Aggregated Aβ may withstand proteolytic degradation [Kuo et al., 1998] and thus seems to be a hard to digest chow for normal microglia [Frackowiak et al., 1992; Paresce et al., 1997]. Moreover, aged glial cells exhibit an impaired phagocytic activity [Yu and McLaurin, 1998]. Thus, in contrast to prevailing beliefs it is posited that, though activated, microglial cells in AD exhibit a suppressed phagocytic activity. Acute ischemia can activate clearing processes in AD patients resulting in the removal of Aβ deposits from ischemic areas compared with the non-ischemic surround [Akiyama et al., 1996; Jendroska et al., 1997]. In ischemic stress and ischemia, Aβ deposition in infarcted and penumbral areas is prevented possibly by similar mechanisms [Jendroska et al., 1995, 1997]. Evidently, the environment in the ischemic area enhances phagocytic activity [Gehrmann et al., 1995] while it appears impaired in the AD tissue milieu.

Macrophages are pleiotropic cells specialized for the recognition, removal and destruction of harmful stimuli. To fulfill their tasks, hundreds of proteins are synthesized and once activated an area of plasma membrane equivalent to their total surface area can be internalized every 35 minutes [Davies, 1984]. Activation of macrophages is a very diverse process. Multiple states of activation exist which require upregulation for one or two complex functions and downregulation of most others, controlled by multiple positive and negative signals [reviewed by Adams, 1994]. It has been estimated that there are at least several hundred different forms of macrophage activation. To reduce unwanted host damage, the type of activation, its duration, and spatial location is precisely controlled by inductive and suppressive signals which balance and countervail each other. Thus, the basic biology of macrophage activation is a yin-yang process, in which on-switches are carefully balanced by off-switches. Of note, mononuclear phagocytes bear upon their surface over 25 receptors for neuroendocrine peptides and hormones and modulation of macrophage-mediated tumoricidal activity by neuropeptides and neurohormones has been shown [Koff and Dunegan, 1985]. Not surprisingly, various behavioural and psychosocial factors can also modulate the activity of macrophages. Particularly, stressful events can impact upon macrophage function [reviewed by Adams, 1994].

There are a variety of candidate factors which may account for the differential phagocytic activity of microglia and astrocytes in AD and ischemia. (1) GC, which are increased in AD (see above) are potent inhibitors of the phagocytosis and oxidative burst of macrophages and astroglia [reviewed by Russo-Marie, 1992; Silvia et al., 1997]. Importantly, suppression of phagocytosis was more pronounced in stimulated macrophages and appeared to affect degradation of ingested material more than ingestion [Grasso et al., 1982; Schaffner and Schaffner, 1987]. The GC-induced inhibition of matrix metalloproteinase expression in neurons, astrocytes and microglia [Shapiro et al., 1991; Gottschall and Deb, 1996] which may affect the degradation of Aβ [Qu et al., 1997; Mentlein et al., 1998] may be involved. In fact, microglial cells in AD cortex, in contrast to microglial cells in the white matter, do not express gelatinase A reactivity [Yamada et al., 1995]. (2) It has been shown in vitro and in vivo that prostaglandins E and E2, produced by activated macrophages, are able to suppress the phagocytic activity of cells from the macrophage lineage in an inhibitory feedback cycle [Hutchison and Myers, 1987; Rossi et al., 1998]. This suppression can be alleviated by cyclooxygenase (COX) inhibitors like indomethacin. Importantly, in brain of AD and Down syndrome patients COX-1, COX-2 and COX-2 mRNA have been found upregulated [Kitamura et al., 1999], particularly in microglial cells [Rollins et al., 1997], in neurons with NFT and damaged axons correlating with the appearance of NFT as well as with ageing [Oka and Takashima, 1997; Lukiw and Bazan, 1997; Pasinetti and Aisen, 1998]. Upregulation of COX-2 mRNA in neurons has been shown as a response to Aβ and excitotoxic stress and paralleled the appearance of apoptosis [Tocco et al., 1997; Pasinetti and Aisen, 1998], though, the study of prostaglandin synthesis in AD brains was inconclusive, showing both increased and decreased production [Iwamoto et al., 1989; Wong et al., 1992]. Since detection of COX-2 mRNA expression and activity is dependent on the post-mortem interval, closely matched samples may further help to elucidate this relation [Lukiw and Bazan, 1997]. (3) Several lines of evidence suggest transforming growth factor-β (TGF-β) as a potent phagocytosis-inhibiting agent. TGF-β inhibits microglial phagocytosis [von Zahn et al., 1997]. TGF-β was found elevated in AD serum and CSF and correlated with disease severity [Chao et al., 1994a, b]. In AD brain, increased TGF-β is associated with SP and tangle-bearing neurons [van der Wal et al., 1993; Flanders et al., 1995]. Accordingly, the amyloidogenic effect of TGF-β

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may be caused by its phagocytosis-mitigating action. (4) In addition, substance P, a potent activator of macrophage phagocytosis [Goldman and Bar-Shavit, 1983; Chanceller-Freeland et al., 1995] is depleted in the AD brains (see above). (5) Furthermore, adhesion and uptake of Aβ is effected through the microglial scavenger receptor [El Khoury et al., 1996; Paresce et al., 1996] which is upregulated in AD [Christie et al., 1996; Honda et al., 1998]. This uptake may be inhibited by competitive binding of cholesterol which has been found elevated in AD brain [Sparks, 1997]. (6) Another potential culprit has been identified recently: IFN activation of microglia decreases phagocytosis rates of Aβ-coated microspheres [Kopec and Carroll, 1997] and inhibits scavenger receptor expression [Geng and Hansson, 1992]. (7) Astrocytes attenuate microglial phagocytosis through a diffusible, yet so far unidentified factor [DeWitt et al., 1998]. Potential candidates are astroglially produced proteoglycans which block Aβ-elicited phagocytic activity [Kopec and Carroll, 1998]. (8) In ischemia, the blood–brain-barrier (BBB) breaks down allowing access of various cellular and humoral factors to the CNS which may activate and assist the microglia in amyloid deposit removal [Akopov et al., 1996]. Based on various lines of evidence, it is speculated that IGF may be such a potential factor: after ischemia a coordinated expression of IGFs by astrocytes and microglia and of IGF receptors by neurons is observed [Gluckman et al., 1993]; additionally, IGF moves into the CNS parenchyma from the CSF and perivascular space [Guan et al., 1996]; IGF is a potent activator of macrophage functions such as phagocytosis, oxidative burst, TNF-α production and killing capacity [Auernhammer and Strasburger, 1995; Renier et al., 1996]. Possibly, the decreased glutamatergic activity of AD may account for the inability to activate the IGF system [Nordqvist et al., 1997]. The role of the breakdown of the BBB for the removal of the SP has been indicated recently. A patient with AD received an omental transposition to improve cerebral blood perfusion [Relkin et al., 1996; Goldsmith, 1997]. Upon autopsy, 31 months after the operation, an intriguing rarefication of SP in the tissue below the omental transposition was found which may be related to the lack of a BBB between the brain tissue and the newly formed vessels originating from the omentum. In contrast, the mild and inconsistent BBB dysfunction and IGF upregulation, but perturbed IGF signal transduction (see above) may be inadequate to counteract the effects of various inhibitors of microglial phagocytosis in AD. Thus, it is suggested that the concomitant abundance of various inhibitors and lack of activators of microglial phagocytosis cause the suppression of deposit clearance typical for AD.

THE FUNCTIONAL AND MORPHOLOGICAL CONSEQUENCES OF PATHOPHYSIOLOGICAL PROCESSES

Progressive cognitive deficits and brain atrophy assessed by imaging techniques are the cornerstones of the clinical diagnosis of AD. The rates of decline correlate strongly with each other [Fox et al., 1999] emphasizing the clinical relevance of cerebral volume loss. Both the loss of neurites and soma contribute to the functional impairment and tissue involution.

Neuronal plasticity and signal transduction systems

The pathophysiological processes cause a widespread disruption of neuronal connectivity and plasticity. Consistently, electron microscopical, immunocytochemical and immunochemical data suggest a substantial loss of synapses and dendritic arborization in AD exceeding the ageing-associated extent [reviewed by Terry et al., 1994; Anderton et al., 1998] which correlates with cognitive impairment and decline [DeKosky and Scheff, 1990; Blennow et al., 1996; Sze et al., 1997]. Analogous to ageing, synaptic size is increased [DeKosky and Scheff, 1990] to compensate for the synapse loss. In contrast to normal ageing, the dendritic arborization declines [Flood and Coleman, 1990]. Structural analysis demonstrated that axonal length correlates with dementia severity in AD, suggesting regressive axonal events as bases of the synaptic pathology while neuron loss or dendritic attrition appeared not to be the primary cause of synapse loss [Anderson, 1996]. This is compatible with findings that in the hippocampus degeneration of presynaptic terminal synaptic boutons precedes, and may lead to the secondary transneuronal degeneration of postsynaptic dendrites [Su et al., 1997]. Importantly, investigations in non-human primate brain suggest that ageing-related synaptic abnormalities antedate the formation of amyloid deposits [Martin et al.,
Another marker of neuronal plasticity, neuronal Na⁺,K⁺-ATPase expression, decreases with the ageing human brain (presumably resulting in a compromise of membrane potential, neuronal excitability and synaptic plasticity) which antedates Aβ deposition and is greatly pronounced in AD brains [Chauhan et al., 1997; Hattori et al., 1998]. On the other hand, the neocortex and hippocampus appear to be in a state of massive somatodendritic sprouting [Ihara, 1988; Jorgensen and Smidt Mogensen, 1997] which may be regarded as an unsuccessful remodelling in response to the synaptic and axonal damage [Scott, 1993]. Overall, the dendritic changes are thought to result from deafferentation-induced plastic changes and degenerative processes secondary to signal transduction failures and cytoskeletal abnormalities [Anderton et al., 1998].

Synapse loss, however, is not specific for AD. It has also been shown in other neurodegenerative disorders such as Lewy body variant of AD [Brown et al., 1998], Pick’s disease [Weiler et al., 1990], Huntington’s disease, Parkinson’s disease and vascular dementia [Zhan et al., 1994] and thus, appears to be the common denominator of cognitive impairment irrespective of the underlying pathogenesis [Heffernan et al., 1998].

A multitude of neurotransmitter systems are affected by AD. A full appreciation of this complex subject clearly exceeds the scope of this paper and only general principles shall be covered [reviewed by Nordberg, 1992; Greenamyre and Maragos, 1993; Francis et al., 1994]. The most consistent changes are found in various features of the cholinergic system [reviewed by Geula and Mesulam, 1994]. Cholinergic innervation of cortex and hippocampus is depleted due to a substantial loss of basal forebrain cholinergic neurons. Acetylcholine (ACh) synthesis assessed by choline acetyltransferase (ChAT) activity is impaired early in the course of the disease. Muscarinic M1 receptors are preserved. Presynaptic M2 receptors were reportedly decreased, unchanged or increased; some loss of M2 receptors, however, may be expected due to the degeneration of basolocortical projections. Nicotinic receptors, on the other hand, are found substantially diminished at autopsy and in vivo and the loss correlates with synaptic pathology. Cholinergic agonist-induced signal transduction is brain region-selectively impaired. These cholinergic deficits as well as CSF ACh concentrations highly correlate with the cognitive impairment in AD.

Equally the serotonergic, adrenergic and glutamatergic systems are involved [reviewed by Palmer and DeKosky, 1993]. Both the noradrenergic locus coeruleus (LC) and serotonergic raphe nucleus are degenerating. Noradrenaline (NA) transporter is decreased in the LC. At this point an intriguing relationship between LC and immune responses should be pointed out. Lesion of the LC induced a variety of immune deficiencies including impaired ability to produce antibodies, and to develop positive Arthus and delayed hypersensitivity skin reactions and experimental autoimmune encephalomyelitis [reviewed by Jankovic, 1994]. Thus, the degeneration of the NA system may contribute to the immunological deficits seen in AD. Serotonergic functions are impaired as well. Cortical 5-HT levels, 5-HT uptake, and stimulated 5-HT release were found reduced. The glutamatergic system is also affected [Maragos et al., 1987; Greenamyre and Maragos, 1993; Olney et al., 1997]. Antemortem cerebral amino acid concentrations indicate selective degeneration of glutamate-enriched neurons in Alzheimer’s disease. The cortex and hippocampus exhibit loss of glutamate binding sites. Predominantly NMDA receptors and their subunits are altered, while either loss or increase of hippocampal AMPA receptors have been reported. Deficient glutamate transport in AD might be involved by failing to clear excess glutamate at synaptic clefts which may result in increased excitotoxicity and synaptic degeneration. In contrast, AD brain levels of dopamine and dopaminergic receptors were not altered.

Consistently, the GABAergic system has been described as least affected [Greenamyre and Maragos, 1993; Davies et al., 1998]. While GABA_A agonist binding in the cortex is unchanged there appear to be area-specific hippocampal decrements of both GABA_A and GABA_B receptors which affect the z1 subunit while β2/3 subunits appear to be preserved [Mizukami et al., 1998].

Intracellular signal transduction systems and their network integration are compromised [reviewed by Horsburgh and Saitoh, 1994; Fowler et al., 1995]. Changes in level and function of G-proteins have been documented. Likewise, the adenyl cyclase system is compromised. Various constituents of the phosphoinositol signalling system, including phospholipase C (PLC) and PKC, were found dysregulated in AD brains [Fowler, 1997]. These changes appear to be highly specific, affecting selected members of the PKC, PLC, G-protein and adenyl cyclase families while
Neuronal demise: apoptosis or necrosis?

Neuronal cell death although widespread in AD brains occurs in a selective pattern with a regional and laminar distribution [Hyman et al., 1995]. Notably, volume and neuronal numbers of hippocampal CA1 correlate uniquely with disease duration and severity [Bobinski et al., 1998]. In general, cell death develops along two pathways: apoptosis and necrosis [reviewed e.g. by Bennett and Huxlin, 1996]. Apoptosis (Greek ‘falling off’) describes the silent demise of cells which occurs during development, cell maturation, and in response to various noxious stimuli [Thompson, 1995]. During apoptosis, neurons lose their synaptic connections [Ivins et al., 1998; Mattson et al., 1998b], neatly package their contents and offer them to neighbouring cells for cellular recycling. In contrast, necrotic cell death results from a severe environmental insult overwhelming the cellular adaptation processes, leading to profound osmotic imbalances with swelling of intracellular organelles. Cell lysis occurs rapidly, accompanied by an inflammatory response. Thus, acuity and severity of the noxious stimuli and dosage of toxic agent will determine the pathway of cell death [Bonfoco et al., 1995; Kroemer et al., 1998], together with intrinsic cellular vulnerabilities [Gschwind and Huber, 1995]. The fundamental difference between necrosis and apoptosis is that necrosis occurs in conditions of depletion of ATP while apoptosis needs some residual ATP due to its energy demands [Eguchi et al., 1997; Nicotera and Leist, 1997].

A wealth of evidence suggests that mitochondria [Kroemer et al., 1998] and alterations in intracellular Ca$^{2+}$ compartmentalization [Nicotera and Orrenius, 1998] play a decisive role in the induction of apoptosis. Even in cell-free systems, mitochondria are required for apoptosis [Kroemer et al., 1998]. Mitochondrial permeability transition (PT) is a critical, rate-limiting event of apoptosis, since its induction suffices to cause apoptosis, and disruption of the mitochondrial transmembrane potential and generation of reactive oxygen species are constant features of early apoptosis. Mitochondrial Ca$^{2+}$ accumulation determines nuclear Ca$^{2+}$ accumulation and DNA fragmentation which can be decreased by inhibition of mitochondrial Ca$^{2+}$ cycling [Faulk et al., 1995].

Recent evidence suggests apoptotic cell death in AD brains [Cotman and Su, 1996]. DNA strand breaks as markers of apoptotic cell death were identified in vulnerable regions of AD brains and correlated with disease progression [Troncoso et al., 1996; Sugaya et al., 1997]. Apoptotic DNA fragmentation was routinely found in NFT-containing cells but was not consistently related to SP [Sugaya et al., 1997].

Expression of the protooncogene bcl-2 is increased in AD brains, predominantly in reactive astrocytes [O’Barr et al., 1990]. In neurons, bcl-2 immunoreactivity increased with disease severity [Satou et al., 1995] and its upregulation was associated with DNA damage [Su et al., 1996]. Bcl-2 is preferentially integrated into the mitochondrial inner membrane and ER membrane and suppresses apoptosis by regulating ER-associated Ca$^{2+}$-fluxes [Lam et al., 1994], nuclear and cytosolic Ca$^{2+}$ and its signalling [Marin et al., 1996; Qi et al., 1997]. Furthermore, it prevents the decrease of mitochondrial transmembrane potential [Shimizu et al., 1996; Satoh et al., 1997], the generation of reactive oxygen species [Kane et al., 1993; Lawrence et al., 1996], and the release of cytochrome c from mitochondria [Kluck et al., 1997]. Moreover, bcl-2 potentiates the maximal Ca$^{2+}$ uptake capacity of neural cell mitochondria [Murphy et al., 1996] and prevents Ca$^{2+}$ influx into mitochondria [Baffy et al., 1993] thereby optimizing and protecting mitochondrial Ca$^{2+}$ and energy homeostasis. Additionally, it may affect the cellular redox potential through upregulation of SOD, CAT and by increasing total glutathion and non-oxidized glutathion [Ellerby et al., 1996] and thus decreases lipid peroxidation [Hockenbery et al., 1993]. Overall, the bcl-2 family of oncogenes regulates apoptosis by either direct or indirect modulation of PT [Kroemer, 1997]. However, the PT-protecting effect of bcl-2 has a limited spectrum, e.g. bcl-2 is unable to counteract high Ca$^{2+}$ levels of 500 µM [Zamzani et al., 1996]. Accordingly, bcl-2 upregulation in AD brains may be regarded as an attempt to evade the detrimental consequences of the disruption of Ca$^{2+}$ homeostasis and may label more resistant, surviving cells.
Intriguingly, Aβ downregulates bcl-2 and upregulates bax, a pro-apoptotic protein, in human neurons, rendering them more vulnerable to oxidative stress [Paradis et al., 1996]. The lower level of bcl-2 in tangle-bearing neurons also indicates an increased vulnerability of these cells [Su et al., 1996]. Hyperphosphorylation may also impair the ability of bcl-2 to inhibit cell death [Haldar et al., 1996]; yet, no information about the state of phosphorylation of bcl-2 in AD brain is available. Several pro-apoptotic regulators have been assessed in AD brain. A member of the bcl-2 family, bax, a pro-apoptotic, was found elevated in AD brains and may precede tangle formation [MacGibbon et al., 1997], while a decreased bax expression was seen in surviving neurons of the hippocampal dentate gyrus [MacGibbon et al., 1997]. Pro-apoptotic p53 was also increased in neurons and glial cells of AD temporal and frontal cortices [de la Monte et al., 1997; Kitamura et al., 1997] and interleukin-1β converting enzyme (ICE)-ζ was overexpressed in AD frontal cortex [Desjardins and Ledoux, 1998].

Other lines of evidence also point at apoptosis as a predominant pathway of cell death in AD. Aβ induces apoptosis in neurons [Loo et al., 1993; Watt et al., 1994; Li et al., 1996; Estus et al., 1997] and apoptosis-related events in synaptosomes and dendrites [Ivins et al., 1998; Mattson et al., 1998b]. Expression of mutated APP causes apoptosis in vitro which is mediated by the G protein Go [LaFerla et al., 1995; Yamatsuji et al., 1996; Giambarella et al., 1997]. Immediate early genes, markers of apoptotic death-related transcription processes [reviewed by Draganow and Preston, 1995; Schreiber and Baudry, 1995] were found increased in AD brain and after Aβ in vitro toxicity [Zhang et al., 1992; Anderson et al., 1994; Cotman and Anderson, 1995]. Excitotoxic cell death is associated with increased APP expression in the apoptotic death pathway, but not in the necrotic pathway [Lesort et al., 1997b]. This APP upregulation may result in an increased Aβ secretion as a uniform response to cellular damage [Galli et al., 1998].

However, other researchers have found predominant necrosis in AD brains [Lucassen et al., 1997, 1998; Stadelmann et al., 1998]. Likewise, in vitro Aβ also induced necrosis rather than apoptosis [Behl et al., 1994]. These conflicting data may, at least partly, be reconciled by the finding that Aβ cell type-dependently either induced apoptosis or necrosis in vitro [Gschwind and Huber, 1995]. As mentioned earlier, the energetic status of the cells determines the cell death pathway. It is suggested that concomitant cerebrovascular pathology resulting in a reduced perfusion (see part III of this series), and even prolonged agonal states could have major impacts on the energetic status of neurons and thus shift the prevailing cell death from apoptotic to necrotic type. This shift may also have important implications for the distribution of markers of oxidative stress. For instance, while bcl-2 upregulates anti-oxidants like glutathione [Ellerby et al., 1996] necrotic lysis tends to predominate under conditions of reduced glutathione [Kane et al., 1993; Fernandes et al., 1994]. Thus, evidence exists that apoptosis and necrosis preferentially proceed at different levels of pro-oxidants and anti-oxidants [reviewed by Slater et al., 1995]. Some of the conflicting data with respect to AD brain redox levels, as discussed previously, may be explained by different tissue samples or pathophysiological factors influencing the apoptosis/necrosis shift [reviewed by Nicotera et al., 1999]. It is also suggested that there may be a range of conditions in which a heterogeneous mixture of cells, like in the brain, may pursue both apoptotic and necrotic pathways at the same time. Even more complicating, changing metabolic conditions may have the consequence that an individual cell after entering the apoptotic pathway may experience more profound metabolic deficits and may have to undergo secondary necrosis. Along this line of shared pathogenetic events, recently an apoptosis-necrosis continuum was defined which characterizes a variety of injurious neuronal cell death paths [Martin et al., 1998]. Finally, recent findings that occurrence of chromatin condensation and DNA ladders are not able to discriminate between apoptosis and necrosis [Bicknell and Cohen, 1995; Gras-Kraupp et al., 1995; Stadelmann et al., 1998], that DNA fragmentation is not required for apoptosis [Cohen et al., 1992; Falcieri et al., 1993; Schulze-Osthoff et al., 1994], and that oxidative stress-induced cell death of nerve cells may not follow the traditional apoptosis/necrosis distinction [Tan et al., 1998], may further relativize the relevance of the apoptosis/necrosis dispute concerning the predominant neuronal death pathway in AD.

Selective vulnerability of neurons

Neuroanatomically, the topography of AD pathology is highly selective, affecting some brain areas and neurons and sparing others. Various aspects of

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this highly complex feature have been reviewed recently [Hof and Morrison, 1994; Van Hoesen and Solodkin, 1994; Morrison et al., 1998]. Given the multifactorial genesis of AD-related loss of synaptic connectivity and cell death, the variables determining cellular vulnerability should be equally complex and only the composite appreciation of the individual variables will give a comprehensive picture of the individual cell’s vulnerability profile. Moreover, the pattern of vulnerability may change. With increasing age at onset, a decreasing predominance of temporoparietal symptoms and neuropathology was found [Risberg et al., 1990; Giannakopoulos et al., 1997].

Neuropathological assessment reveals a spatial and temporal sequence of events. The staged course is characterized by the sequential involvement of the basal forebrain, entorhinal cortex, hippocampus, amygdala, and association cortical areas [Braak and Braak, 1991, 1997]. Evolutionary, these regions have in common a massive expansion, due to the disproportionate hominid enlargement of the association cortex and of functionally linked regions [Rapoport, 1990; Neill, 1995]. Functional brain imaging reveals a higher degree of AD-related metabolic compromise in these limbic-cortical neuronal circuits [Stein et al., 1998]. All neurons at risk of NFT formation are large pyramidal cells that send long projections to other brain areas [Morrison et al., 1998]. While in general neuronal size may be a marker for degeneration [Dani et al., 1997], large pyramidal hippocampal CA3 neurons appear to be relatively resistant to neurodegeneration indicating that features other than merely cell size and morphology determine vulnerability [Morrison et al., 1998]. The differential, ageing- and AD-specific susceptibility of neurons from subcortical areas which form the ‘reticular core’ may be related to the degree of retained capacity for dendritic remodelling [Arendt et al., 1998]. These capacities may depend functionally on distinct protein kinase equipment [McKee et al., 1990], high levels of neurofilament proteins [Hof and Morrison, 1994; Morrison et al., 1998], tau isoform profiles and tau mRNA expression under cellular stress [Bahr and Vicente, 1998; Delacourte et al., 1998; Esclaire et al., 1998] which are markers of neurons destined to degenerate in AD.

A variety of other neurobiological markers for the differential vulnerability of nerve cells has been described. As an early event, the endosomal–lysosomal system is markedly activated in vulnerable neuronal populations as evidenced by an upregulation of various cathepsin proteinases [Cataldo et al., 1998]. The ability to cope with a given Ca²⁺ load may determine the susceptibility to excitotoxic, oxidative and metabolic stress-induced damage. Thus, neurons are differently endowed to cope with Ca²⁺ overloads [Mills and Kater, 1990]. Basal forebrain neurons, for instance, possess a large Ca²⁺ buffering capacity but the rate of Ca²⁺ clearance is relatively small in relation to the Ca²⁺ influx, exposing the cell to potentially neurotoxic increased Ca²⁺ levels for extended periods [Tatsumi and Katayama, 1995]. Developing cerebellar granule cells, on the other hand, are resistant to glutamate [Balazs et al., 1988; Hack et al., 1993] and need plasma membrane depolarization and sustained intracellular Ca²⁺ concentrations for survival [Koike and Tanaka, 1991; D’Mello et al., 1993]. As an increased activity of the Na⁺/Ca²⁺ exchanger has been found in post-mortem AD neurons from the frontal and temporal cortex but not from cerebellum, this feature may confer a selective survival advantage to otherwise endangered nerve cells [Colvin et al., 1994]. Of note, the ability to downregulate/inactivate Ca²⁺ channels or handle cytosolic Ca²⁺ more effectively and thus become more resistant may be acquired after a preceding insult [Bickler and Buck, 1998; Shimazaki et al., 1998] and the inability to achieve such compensatory changes (e.g. to upregulate calbindin D28k) would be a vulnerability factor [Rami et al., 1998]. Another important susceptibility factor may be the type of Ca²⁺-binding protein expressed by the cells [McBurney and Neering, 1987]. Calbindin appears to be a marker for vulnerable neurons (see above). Cultured GABAergic as well as calretinin-immunoreactive neurons are resistant to toxicity induced by Aβ and Ca²⁺ overload [Lukas and Jones, 1994; Pike and Cotman, 1995]. In AD cortex, calretinin-containing neurons appear to be resistant to neurofibrillary pathology and degeneration [reviewed by Morrison et al., 1998]. Similarly, in the AD hippocampus calretinin-positive in contrast to calbindin-positive neurons are free of PHF [Ikonomovic et al., 1998]. Parvalbumin-immunoreactive neurons exhibit a differential susceptibility with interneurons being resistant in the cortex but partially vulnerable in the hippocampus and entorhinal cortex [Solodkin et al., 1996; Brady and Mufson, 1997; Ikonomovic et al., 1998]. This differential susceptibility may implicate important functional differences of the neuronal populations.
Resistance to degeneration may be associated with expression of nitric oxide synthase [Simic et al., 1997; Lucassen et al., 1998], the pathophysiological relevance of which will be discussed in part IV of this series. A feature which may implicate lipid homeostasis in the pathophysiological process (extensively discussed in part III of the series) is the histological finding of a selective survival of neurons which express clusterin (apo J), an apoprotein which has been reported to protect against Aβ toxicity and the complement attack complex [McGeer et al., 1992; Boggs et al., 1996]. The broad range of differential vulnerabilities of neurotransmitter cell populations (see above) is epitomized by cholinergic basal forebrain (see below) and acetylcholinesterase-rich neocortical and hippocampal pyramidal neurons [Mesulam and Geula, 1988; Beeri et al., 1995] on one end and GABAergic neurons [Davies et al., 1998] on the other end of the spectrum. Cell-specific resistance to oxidative stress due to coordinated changes of antioxidant pathways may be another source of differential vulnerability [Sagara et al., 1998]. Evidence suggests that the entire AD brain is subject to oxidative challenge, however, brain areas and cell population are differentially susceptible. An increased antioxidant enzyme activity may protect against Aβ toxicity [Sagara et al., 1998]. Neuronal populations which are vulnerable to degeneration may show higher levels of constitutively expressed SOD [Delacourte et al., 1988; Bergeron et al., 1996]. On the other hand, the increased vulnerability of aged neurons to ischemic insult was correlated with a reduced stimulated expression of SOD [Zhai et al., 1990]. Thus, a high basal SOD expression combined with a reduced stimulated response may predispose neurons to oxidative vulnerability. This may coincide with a higher metabolic activity since there is a higher level of gene expression of the oxidative phosphorylation complex in vulnerable neurons of the temporal cortex as compared with the motor cortex [Fukuyama et al., 1996]. The transcription factor NF-κB may be another resistance marker. NF-κB which has been found elevated in affected brain areas [Schwarz et al., 1997], is more active in neurons resistant to Aβ toxicity but suppression of NF-κB activity reverses this resistance [Lezoualc'h et al., 1997]. The relative abundance of pro-apoptotic and anti-apoptotic members such as p53 [Xiang et al., 1996], bcl-2 and bax (see above) may be the final regulator of cell death susceptibility of neurons. Thus, at last the intrinsic programming of the cell death machinery determines the vulnerability of the neurons [McConkey and Orrenius, 1996].

The multifactoriality of the selective susceptibility is highlighted exemplarily with regard to the neurons of the basal forebrain, entorhinal cortex, and hippocampus which undergo preferential damage during the disease process [Whitehouse et al., 1982; Coyle et al., 1983; Hyman et al., 1984]. The early involvement of parahippocampal structures can be exploited for diagnostic purposes. Atrophy of the hippocampal formation and medial temporal lobe allow an early in vivo differentiation between normal aged and incipient AD patients with imaging techniques [Fox et al., 1996; Detolio-Morrell et al., 1997; Jack et al., 1999]. In the hippocampus, specific regions (layers CA1 and CA3) are particularly vulnerable to a variety of disorders such as ischemia, trauma, epilepsy, and AD, while other regions (dentate gyrus and CA2) are more resistant to acute and chronic neurodegeneration [Maragos et al., 1987; Mattson et al., 1989]. Neurons in the vulnerable regions are characterized by the ageing-related modulation of a variety of hormone receptors, e.g. IGF-1 and GC receptors [see Heininger, 1999], high expression of glutamate receptors [Mattson et al., 1989; Thorns et al., 1997], ageing-related increase of L-type Ca2+ channel density [Thibault and Landfield, 1996], various abnormal features of the Ca2+-phosphate signalling cascade [McKee et al., 1990], increased basal and stimulated APP expression [Hall et al., 1995; Thorns et al., 1997], under cellular stress preferential expression of APP containing the Kunitz-type protease inhibitor [Johnson et al., 1990; Abe et al., 1991; Panegyres, 1998], selective internalization of Aβ peptides [Bahr et al., 1998], increased postsischemic apoE expression [Hall et al., 1995], and metabolic vulnerability [Davolio and Greenamyre, 1995; Geddes et al., 1996, 1997; Ajilore and Sapolsky, 1997]. Enhanced vulnerability to excitotoxicity in hippocampal neurons occurs with age in culture [Geddes et al., 1997] possibly associated with increased Ca2+ transients [Thibault et al., 1997]. The hippocampal areas are also remarkable for ageing-related upregulation of heat shock protein expression [Tohgi et al., 1995] which are a marker of relative resistance [States et al., 1996] but furthermore indicate a general metabolic stress in these regions. Intriguingly, the acuity of the energetic compromise may affect the pattern of neuronal vulnerability: acute metabolic disruption created an ischemia-like pattern of
predominant CA1 damage while chronic impairment led to AD-like changes with CA3/CA1 degeneration [Geddes et al., 1996].

Cholinergic basal forebrain neurons exhibit a variety of susceptibilities to damage by excitotoxins, nitric oxide and free radicals [Harrington and Wenk, 1992; Wenk, 1995]. Loss of target neurons in the hippocampus deprives these neurons of retrogradely transported neurotrophins and renders them vulnerable [Cooper and Sofroniew, 1996]. Moreover, these neurons exhibit various dependencies on thyroid and gonadal hormone supply [see Heininger, 1999]. Loss of ovarian function results in the significant decrease of cholinergic markers in the basal forebrain [Gibbs, 1998]. Basal forebrain cholinergic neurons are markedly vulnerable to AMPA/kainate excitotoxicity [Weiss et al., 1994]. Moreover, during ageing basal forebrain and hippocampal neurons gradually upregulate AMPA receptors [Geddes et al., 1992; Jasek and Griffith, 1998] which exhibit an increased Ca\(^{2+}\) permeability with ageing and thus put the neurons under increased stress [Pagliusi et al., 1994; Ikonomovic and Armstrong, 1996; Akaike and Rhee, 1997]. Such mechanisms may underlie the heightened vulnerability of aged basal forebrain neurons to excitotoxicity [Zawia et al., 1992; Waters and Allen, 1998]. Remarkably, similar processes appear to take place after oxygen–glucose deprivation [Ying et al., 1997]. Cholinergic stimulation is neurotoxic for nucleus basalis cells in the presence of a mitochondrial energy deficit [Wenk et al., 1996]. Cholinergic cells are selectively vulnerable to A\(\beta\) toxicity [Harkany et al., 1995; Diaz et al., 1996] and are selectively killed by activated microglial cells in basal forebrain mixed neuronal/glial cultures [McMillian et al., 1995]. Since neurotrophins inhibit the activation of microglia [Neumann et al., 1998], the lack of neurotrophins in the aged basal forebrain and hippocampus may add to the evolution of an inflammatory response. To the same end may contribute the expression of lipoxynenase, a key enzyme in the synthesis of inflammatory eicosanoids, which is upregulated in vulnerable neurons of the aged brain [Uz et al., 1998]. There may also be a reduced participation of intracellular organelles like mitochondria and ER in the buffering of Ca\(^{2+}\) in septal neurons rendering the neurons more dependent on the action of Ca\(^{2+}\)-binding proteins [Blandman et al., 1993]. Indeed, Ca\(^{2+}\)-binding proteins confer an increased resistance to metabolic challenge [Meier et al., 1997], but are reduced in cholinergic basal forebrain and hippocampal cells during ageing [Heininger, 1999] and further in AD (see above).

In part III of this series a variety of established and putative risk factors and their interaction with the basic pathophysiological processes and the relative significance of genetic and environmental risk factors will be discussed.

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