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Amyloid β-Peptide and Oxidative Cellular Injury in Alzheimer's Disease

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Abstract

Alzheimer's disease is a progressive neurodegenerative disorder that affects primarily learning and memory functions. There is significant neuronal loss and impairment of metabolic functioning in the temporal lobe, an area believed to be crucial for learning and memory tasks. Aggregated deposits of amyloid β-peptide may have a causative role in the development and progression of AD. We review the cellular actions of Aβ and how they can contribute to the cytotoxicity observed in AD. Aβ causes plasma membrane lipid peroxidation, impairment of ion-motive ATPases, glutamate uptake, uncoupling of a G-protein linked receptor, and generation of reactive oxygen species. These effects contribute to the loss of intracellular calcium homeostasis reported in cultured neurons. Many cell types other than neurons show alterations in the Alzheimer's brain. The effects of Aβ on these cell types is also reviewed.

Index Entries: Excitotoxicity; nerve; astrocyte; microglia; endothelial cell; ion-motive ATPase; free radical; signal transduction.

Introduction

Amyloid β-peptide (Aβ) is the primary component of the neuritic plaques that are a hallmark of Alzheimer's disease (AD). Reviews have been written about the protein processing pathways that can lead to the production of Aβ and about the aggregation properties and toxicity of the peptide *(1,2)* but only recently have reports begun to address the mechanism of Aβ-induced neurotoxicity (*3–5*, and references cited therein). Excitotoxicity, free radicals, and reduced trophic support have all been postulated to play roles in AD *(4–6)*. In this article, we will consider that many of the observed changes in AD can be attributed to the direct actions of Aβ or to an Aβ-induced cascade of events.

Several lines of evidence point to Aβ having a primary role in the etiology of AD. Molecular genetic studies have linked several mutations with cases of familial AD *(1,7,8)*. Although familial AD accounts for only about 5–10% of the cases of AD, studying these cases can yield

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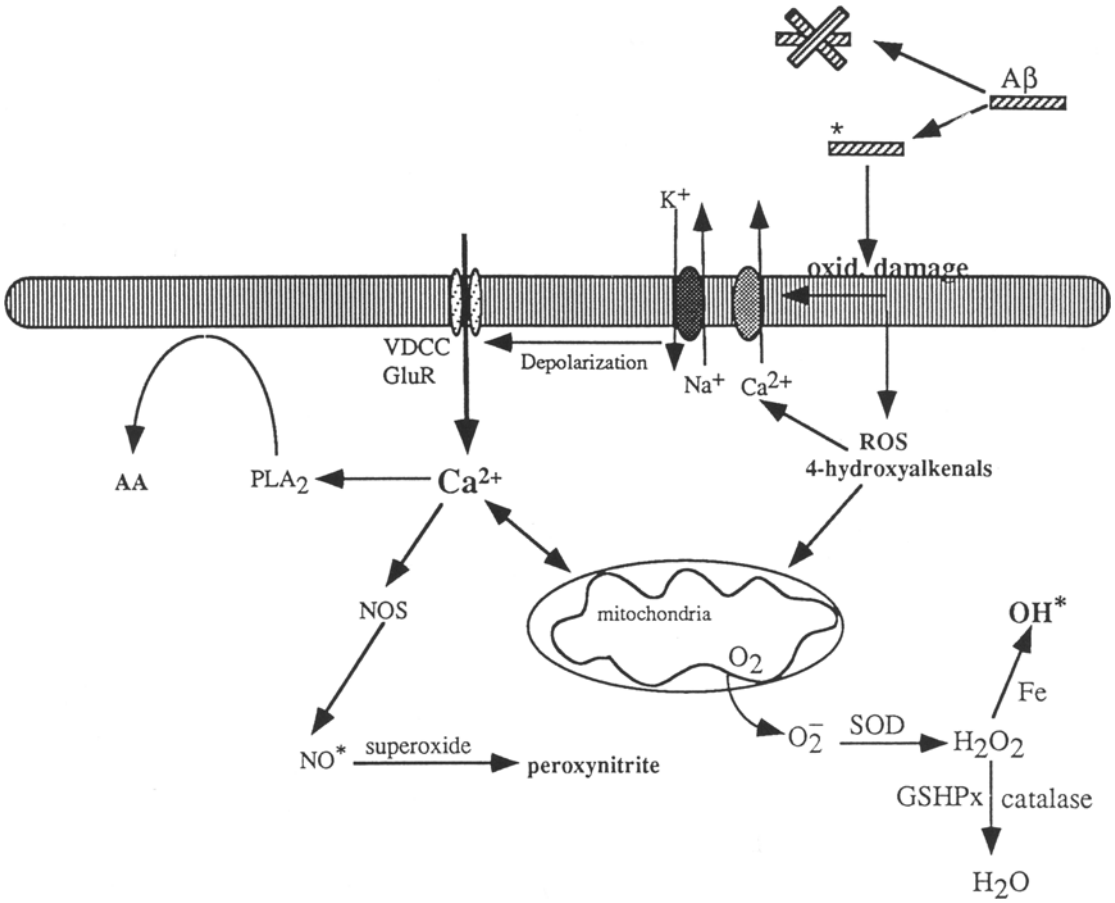

Fig. 1. Schematic diagram depicting possible mechanisms of Aβ's actions in neurotoxicity. Aβ-induced lipid peroxidation impairs plasma membrane ion-motive ATPases. This leads to depolarization and subsequently glutamate release and the activation of voltage-dependent calcium channels. The resulting increase of $[Ca^{2+}]_i$, as well as the lipid peroxidation, can activate PLA_2, which will release arachidonic acid from the membrane. The increase in $[Ca^{2+}]_i$ can also cause the mitochondria to take up high concentrations of Ca^{2+}. This results in uncoupling of the electron transport chain and an increase in the release of superoxide anion. The NO produced by the activation of NOS can react with superoxide anion to produce highly reactive and toxic peroxynitrite.

clues to AD in general. A common characteristic of genetic linkages to AD that have been identified is increased Aβ production and/or increased production of forms of Aβ that show increased aggregation properties. In addition, transgenic animals that overexpress mutant human βAPP demonstrate increased neuritic plaques and other pathological changes characteristic of AD *(9)*.

Evidence has been mounting for a role for free radicals in Aβ-induced toxicity. Using cell-culture models, several groups have reported on Aβ-induced increases in intracellular peroxide levels *(4,10)* and increased protein and lipid oxidation *(4,11,12)*. Aβ appears to have its action at the plasma membrane with little if any of the aggregated peptide making its way into the neurons *(13)*. We believe that it is free radical-mediated events initiated at the cell surface that activate a cascade of intracellular events that ultimately lead to neurodegeneration (Fig. 1). Aβ can impair selective plasma membrane ion-motive pumps and increase neuronal sensitivity to excitotoxicity and glucose

withdrawal *(5,13,14)*. An additional alteration that may contribute to disruption of ion homeostasis is damage to a specific type of K^+ channel *(15)*.

Basic fibroblast growth factor (bFGF) and tumor necrosis factor (TNF) have been reported to protect against Aβ toxicity in vitro *(13,16)*. Although the mechanisms of how these growth factors afford protection have not been worked out in each case, the mechanisms that have been determined all pertain to enhancement of the cell's antioxidant defenses or a more direct effect on intracellular calcium levels. For example, bFGF increases antioxidant enzyme levels *(17)*, TNF increases calbindin levels *(18)*, and secreted forms of the amyloid precursor protein (sAPP) activate a K^+ channel, which leads to hyperpolarization of the cell *(19)*.

The actions of Aβ are not limited to effects on neurons. Aβ was shown to impair glutamate uptake in astrocytes *(20)*, disrupt barrier function and glucose uptake in endothelial cells *(21)*, and cause toxicity to vascular smooth muscle cells *(22)*. These reports are examples of the increasing evidence that Aβ may have effects in AD beyond actions on neural cells (Fig. 2).

The Free Radical Nature of Aβ

Several recent reports demonstrate that Aβ stimulates an increase in reactive oxygen species (ROS) within cells. In cultured neurons, Aβ treatment has been shown to increase intracellular peroxide levels and membrane lipid oxidation *(4,10,11)*. The aggregation state of the peptide has been correlated with its toxicity *(23)* and the aggregation of the peptide is dependent on oxygen and is enhanced by oxidizing elements *(24)*. Recent reports by Hensley et al. *(25)* described a unique chemistry of Aβ that involves the peptide itself being a generator of free radicals. Using electron paramagnetic resonance (EPR) spectroscopy, they showed that the peptide reacts with a PBN-adduct via a free radical mechanism in which Aβ(25–35) was the source of the free radical. Just as peptide aggregation was correlated with neurotoxicity, these researchers correlated peptide free radical production with neurotoxicity. However, the peptide demonstrates maximal toxicity during the period of maximal rate of aggregation. In other words, it is possible for the peptide to overaggregate, at which point it displays decreased EPR spectrum and poor toxicity, but still exhibits altered sedimentation in sucrose gradients, shifted electrophoretic migration patterns on gels, and staining with Congo red *(25–27)*.

Free radical generation, *per se*, may not be sufficient to mimic the specific toxic effects of Aβ because some control peptides also generate EPR-detectable radical signals (although with a different profile than Aβ), yet display no neurotoxicity, no effect on $[Ca^{2+}]_i$, and no effect on membrane ion-motive ATPases *(5,13,25)*. Also, treatment of human and gerbil synaptosomes with iron (a strong inducer of oxidative damage) yields effects that differ somewhat from those of Aβ. For example, Aβ treatment reduced the paramagnetic signal of a membrane-bound 12-nitroxyl stearate spin probe and had no effect on a 5-nitroxyl stearate probe, suggesting that Aβ induces oxidation deep within the lipid bilayer. Iron, on the other hand, had little effect on the 12-NS probe but rapidly reduced the signal from the 5-NS probe *(11)*. Treatment of human hippocampal synaptosomes with Aβ(25–35) specifically impaired the plasma membrane Ca^{2+}-ATPase and the Na^+/K^+-ATPase activities with no effect on other membrane ATPases. On the other hand, iron significantly impaired all classes of membrane ATPases *(5)*.

Aβ Effects on Membranes

The loss of intracellular ion homeostasis appears to play a role in the toxicity of Aβ. Aβ can affect the proteins responsible for the maintenance of the ion homeostasis. For example, in cultured hippocampal neurons, an early event in Aβ toxicity is impairment of the Na^+/K^+-ATPase, which preceded increases in intra-

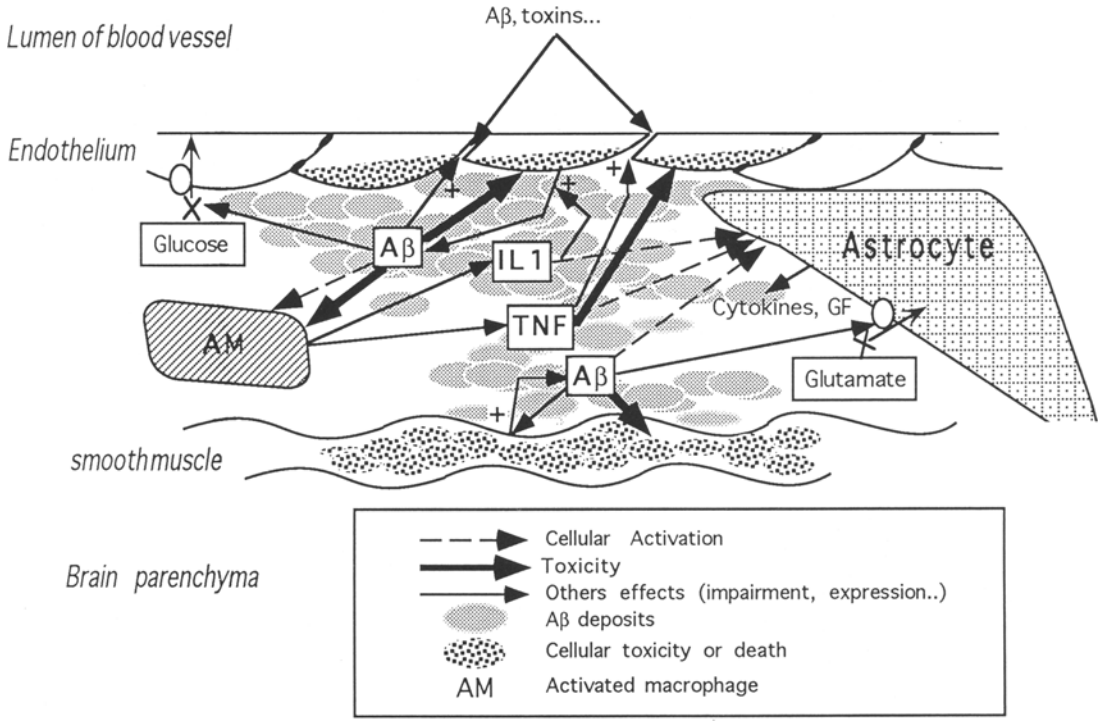

Fig. 2. Cellular actions of cerebrovascular deposits of Aβ. This schematic diagram represents the putative effects of Aβ deposits on the key cell types constituting the cerebral vessels and the blood–brain barrier. Endothelial cells and vascular smooth muscle cells are both capable of expressing βAPP. Cleavage of βAPP by β-secretase leads to Aβ production and deposition. The effects of Aβ can be distinguished as cellular activation and cytotoxicity. Aβ induces activation of astroglial and microglial cells *(90)*. Activated macrophages have been shown to release a number of cytokines, including IL-1 and TNF. These two cytokines, as well as Aβ itself, could in turn activate astrocytes, i.e., could induce astrocyte proliferation and further release of cytokines (e.g., TNF) and growth factors (e.g., bFGF). TNF and subtoxic levels of Aβ, released in the vicinity of endothelial cells, increase their permeability to macromolecules *(21,91)*. This disruption of endothelium integrity could contribute to the entry of serum proteins that are detected in neuritic plaques in AD. Furthermore, higher levels of Aβ and TNF are toxic to endothelial cells. Aβ cytoxicity is mediated by the disruption of intracellular Ca^{2+} homeostasis, the generation of free radicals, and the impairment of energetic metabolism via the inhibition of glucose uptake. IL-1, released by activated microglia, enhances βAPP mRNA expression in endothelial cells, and could thus initiate a vicious cycle leading to increased Aβ depositions. This degenerative process could also be induced directly by Aβ, which is toxic to vascular smooth muscle cells and stimulates its own production in the same degenerating cells. Astrocytes are relatively resistant to Aβ-induced cytotoxicity. Nevertheless, Aβ, via the generation of free radicals, inhibits the glutamate uptake in astrocytes. This effect could enhance the extracellular glutamate concentration and render the neurons more vulnerable to excitotoxicity.

cellular calcium. The impairment of ATPase activity appeared to involve free radicals, because it was blocked with antioxidant compounds.

Various intracellular second messenger systems have been reported to regulate the activities of the Na^+/K^+- and Ca^{2+}-ATPases *(28,29)*. These proteins have also been shown to be exquisitely sensitive to the composition of their lipid environment, and oxidative damage to the lipid membrane has been shown to impair their activities. Wood et al. *(30)* reported that oxidation of as little as 5% of synaptic membrane cholesterol in mice caused a 50% reduction in the plasma membrane Ca^{2+}-ATPase activity. The activity of the Na^+/K^+-ATPase

was unaffected even after oxidation of 40% of the membrane cholesterol. Kutryk et al. *(31)* reported impairment of the Ca^{2+}-ATPase of the smooth endoplasmic reticulum by oxidized cholesterol; the Na^+/Ca^{2+} exchanger was also rapidly impaired and 5 m*M* H_2O_2 was needed to see a similar effect to that seen with cholesterol oxidase. Whether cholesterol oxidation plays a role in AD is not known; however, increasing plasma membrane cholesterol oxidation increases membrane fluidity. There are a number of reports that membranes from AD patients show a decreased fluidity and a decrease in structural order, both of which are characteristics of increased oxidation of the fatty acid side chains of the lipid bilayer, not cholesterol. Subbarao et al. *(32)* looked at basal levels of lipid peroxidation in the frontal cortex and cerebellum in AD patients vs control patients. They found increases in thiobarbituric acid (TBAR) fluorescence in the frontal cortex in AD patients, but no differences between the two groups in the cerebellum. Magnetic resonance spectroscopy studies of brains of AD patients and age-matched controls revealed alterations in phospholipids consistent with membrane oxidation and altered membrane structure *(33)*. It is interesting that in AD, neuritic deposits of Aβ are found in the frontal cortex and hippocampus, areas that show increased membrane oxidation, whereas only diffuse plaques are reported in the cerebellum and there is no increase in oxidative damage to those neuronal membranes.

Another reported effect of Aβ is that it enhances neuronal sensitivity to glutamate toxicity *(13,14,34)*. The mechanism whereby it does this is not known, but Miller et al. *(35)* reported that treatments that decrease membrane fluidity also increase the open state probability of the NMDA channel. Inhibition of the Na^+/K^+-ATPase would be expected to depolarize the membrane, which would relieve the Mg^{2+} block of the NMDA receptor. This will increase neuronal excitability to basal levels of extracellular glutamate and contribute to excitotoxicity. There is some discrepancy in the literature regarding the protection afforded by

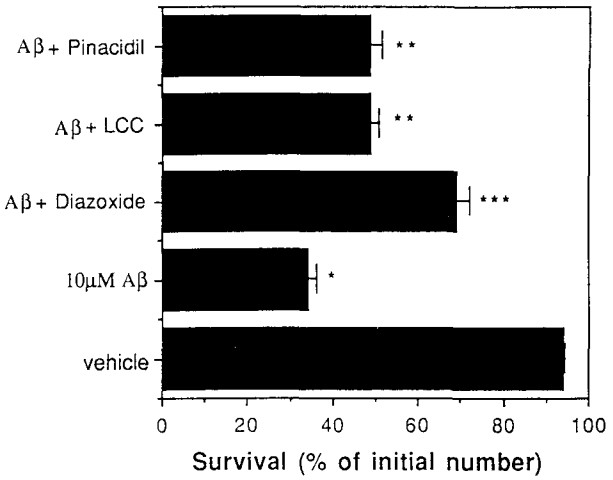

Fig. 3. K^+ channel agonists protect hippocampal neurons from Aβ toxicity. Cultures were pretreated with pinacidil (100 μ*M*), levocromakalim (LCC) (100 μ*M*), diazoxide (1 m*M*), or vehicle (control). Cultures were then exposed to Aβ (10 μ*M*) for 24 h at which time neuronal survival was determined. $*p < 0.001$ compared to vehicle treated; $**p < 0.05$ compared to Aβ alone; $***p < 0.01$ compared to Aβ alone. ANOVA with Scheffe's *posthoc* test.

glutamate receptor and calcium channel antagonists *(13,36,37)*. Weiss et al. *(36)* reported protection with Ca^{2+} channel blockers, but not glutamate receptor antagonists, whereas Busciglio et al. *(37)* saw no protection with either class of compounds. We observed modest protection with glutamate receptor antagonists and removal of extracellular calcium *(13)*. The differences in these results may be a result of differences in the type of neurons used (cortical *[36,37]* or hippocampal *[13]*); species of the neurons (human *[37]* or murine *[13,36]*); as well as differences in culture conditions and experimental treatment methods. That disruption of ion homeostasis is a key component to Aβ-induced neurotoxicity is suggested by the neuroprotective effect of anticonvulsants *(38)*. By keeping the cell hyperpolarized, these compounds are able to offset the depolarizing effect of impairment of the Na^+/K^+-ATPase and thus attenuate the resulting influx of extracellular calcium (Fig. 3). Finally, Aβ can impair mitochondrial function *(39)* presumably by disrupting transmembrane potential in that organelle.

Effects of Aβ on mitochondria occur relatively rapidly (minutes) and also occur with subtoxic levels of the peptide. An important area for future research is to identify the mechanism of mitochondrial impairment induced by Aβ, its relationship to cell death, and its links to Aβ actions at the plasma membrane.

Aβ Alters Signal Transduction Pathways

That there is a deficit in the cholinergic system in the Alzheimer's brain has been known for many years. Numerous therapeutic approaches have attempted to overcome this deficit by inhibiting acetylcholine esterase or otherwise increasing the amount of acetylcholine in the synaptic cleft. Recent reports have called into question the effectiveness of such strategies *(40,41)*. Crews et al. *(40)* found that muscarinic and serotonergic receptors are uncoupled from phospholipase C in cortical membranes from AD patients. Also, Kelly et al. *(41)* reported that Aβ causes an uncoupling of the muscarinic receptor from its GTP binding protein in rat cortical membranes. The latter study showed that free radicals mediated damage to the muscarinic receptor–GTP binding protein system. Therefore, increasing levels of acetylcholine in synaptic clefts may prove ineffective at reversing the cholinergic deficit if the defect is downstream of acetylcholine binding its receptor.

Sato and Sato *(42)* reported that cholinergic nerves originating in the nucleus basalis of Meynert and septal areas, on stimulation, release acetylcholine in the hippocampus and cortical areas. This results in a vasodilatation that increases cerebral blood flow to these areas. Given that Aβ can impair the acetylcholine signaling pathway, one could postulate two effects of deposits of Aβ. First, by uncoupling the muscarinic receptors, Aβ would decrease the signal to stimulate vasodilatation and would thereby attenuate the increase in cerebral blood flow that normally occurs on cortical stimulation/activity. Second, uncoupling of the muscarinic receptor may have presynaptic effects as well. By failing to activate the postsynaptic membrane actively, the cholinergic fibers would not get the trophic signals that come from a correctly functioning synapse. As a result, the synapse may be lost. Diminishing synaptic activity could cause the nerve cell body located in the nucleus basalis/septal area to atrophy.

Nitric oxide (NO) has many reported functions in the CNS *(43)*. The role of NO production in neurotoxicity is receiving increasing attention. Hu and el-Fakahany *(44)* reported that Aβ(25–35) stimulated the production of NO in a neuroblastoma cell line. Sato et al. *(45)* reported a dose-dependent inhibition of the Na^+/K^+-ATPase by sodium nitroprusside (SNP), an activator of NOS. The SNP-induced impairment of the Na^+/K^+-ATPase was attenuated by superoxide dismutase. The above results suggest that impairment of protein function by nitration may play a role in Aβ-induced toxicity. Nitration could occur on and impair growth factor receptors, cytoskeletal elements, and the plasma membrane ion-motive pumps.

Aβ and Damage to Vascular Cells in AD

Cerebrovascular deposits of Aβ and microangiopathy are important hallmarks of AD. Cerebral microvasculature alterations have been observed in AD brains *(46)*. This microvasculature pathology seems to be correlated with AD lesions *(47–49)*. Decreased density, smoothness, and thickness of capillaries have been observed in vulnerable brain regions like layers III and IV of superior frontal cortex, inferior temporal cortex, subiculum, and area CA1 of the hippocampus *(46)*. Degenerating cerebral endothelium *(50)* and vascular smooth muscle *(51)*, and a vacuolized vascular basement membrane have been described, principally in vulnerable brain regions of AD like Broadman cortical area 22 *(52)*. Furthermore, proteins from the vascular basement membrane like heparin sulfate proteoglycan, collagen type IV, fibronectin, laminin, or serum

proteins, like amyloid P, immunoglobulins, or complement factors, are often associated with plaques *(52)*. A compromised integrity of the blood–brain barrier in AD has been suspected to be responsible for these depositions and for the entry into the central nervous system (CNS) of toxins *(48)*. Cerebral Aβ accumulations in plaques have even been suggested to result from a peripheral source. Maness et al. *(53)* showed the accumulation of iodinated Aβ in brain parenchyma and cerebrospinal fluid after iv injection. A great variety of peripheral cells are able to synthesize Aβ, like vascular smooth muscle cells, platelets, megakaryocytes, fibroblasts, endothelial cells, or lymphocytes *(22,54–56)*. In addition, sequences of vascular and plaque amyloids are very similar *(57,58)*. Nevertheless, the hypothesis of the peripheral origin of Aβ is controversial, since Frautschy et al. *(59)* demonstrated that Aβ injected in rat brain could be detected after a month in the vicinity of cerebral blood vessels.

Since Aβ exerts deleterious effects on neuronal cells and microvascular alterations have been reported in AD, one could expect Aβ to be toxic to vascular cells. Indeed, Aβ induces the degeneration of cerebrovascular smooth muscle cells in vitro and stimulates its own production in these cells *(22)*. Endothelial cells represent one of the key cell types involved in the blood–brain barrier function and integrity. Degenerating cerebral endothelium *(50)*, diminished mitochondrial density, increased number of pericytes per vessel in AD brain *(60)*, and impairment of the glucose transporter *(61)* have all been described in the microvasculature of AD brain, and are suggestive of an increased permeability of the blood–brain barrier.

Recent data suggest the possibility that Aβ promotes degeneration of the cerebrovascular endothelium in AD. Thomas et al. *(62)* reported that Aβ can damage and kill endothelial cells in an *ex vivo* blood vessel ring preparation; the damage was prevented by superoxide dismutase, indicating the involvement of oxyradicals. We investigated whether the Aβ cerebrovascular deposits may be somehow related to these endothelial abnormalities. We showed that Aβ(25–35) and Aβ(1–40) are toxic to cultured endothelial cells in vitro at concentrations that are also toxic to neuronal cells *(21)*. This Aβ-induced cell death is apoptotic, as evidenced by the blocking effect of RNA and protein synthesis inhibitors, such as cycloheximide and actinomycin D, as well as by DNA fragmentation and condensation observed after Hoechst staining *(21)*. Furthermore, a subtoxic level of exposure to Aβ increased endothelial cell permeability to a macromolecule, albumin, and impaired glucose uptake by the endothelial cells. Since these effects were all blocked by agents inhibiting the intracellular Ca^{2+} increase and by antioxidant compounds, Aβ-induced endothelial cell permeability, glucose uptake impairment, and apoptosis may involve free radical generation and intracellular Ca^{2+} homeostasis dysregulation, a cellular mechanism similar to that described in neurons *(5)*. Thus, the putative leakiness of the blood–brain barrier and the impairment of the glucose transporter observed in AD brain microvessels *(61)* could be mediated by cerebrovascular deposits of Aβ. Endothelial cells, together with vascular smooth muscle cells, can express β-amyloid precursor protein (βAPP) *(22,56)*. In endothelial cells and neurons, interleukin 1 (IL-1) enhances the expression of βAPP and may thus contribute to the increase in peripheral and cerebral accumulation of Aβ *(56,63)*.

Aβ Effects on Glial Cells in AD

Aβ has been shown to affect glial cells in several different ways. For example, Aβ enhanced glial production of IL-1 *(64)*. IL-1 is expressed by microglia in the CNS *(64)*, and elevated levels of IL-1 have been found in AD *(64–67)* and in Down's syndrome *(64)*, particularly in microglia *(65–69)*. Activated astroglia and microglia are often associated with neuritic plaques. Culture studies suggest that Aβ itself may be responsible for the astroglia and microglia activation *(64,70,71)*. IL-1 stimulated proliferation of astrocytes *(69)*. Aβ exposure induces

increases in complement C3 component *(72)* and IL1β *(73)*, which protects cortical neurons against excitotoxicity *(69)* and exerts neurotrophic actions on hippocampal neurons in culture *(74)*. In addition, activated microglia have been shown to express several other cytokines, like tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β). These two cytokines protect cultured hippocampal neurons against Aβ-induced toxicity *(16,75)*. However, process-bearing, ramified microglia cells are selectively vulnerable to Aβ exposure compared to nonprocess-bearing and ameboid forms of microglia *(76)*. Additional data indicate that Aβ promotes an increase in resting intracellular concentration of Ca^{2+} in microglia, in both ameboid and ramified cells *(77)*. This Ca^{2+} rise may precede microglial changes like phagocytosis and oxygen radical release. The release of protective cytokines by activated microglia in the vicinity of cerebral Aβ deposits suggests a beneficial role for activated microglia in the pathogenesis of AD. However, activated microglia also release neurotoxic substances, like ROS or excitotoxins *(78)*. Microglia can thus exert opposing effects on neuronal survival. The balance between these different functions might influence the vulnerability of neurons in AD. Nevertheless, in a recent study, we observed that damage to neurons after ischemic or excitotoxic insults were increased in transgenic mice lacking TNF receptors compared to the wild-type mice. In this study, microglia activation was greatly reduced *(79)*. This raises the possibility that the protective role of microglia activation may be more important than the potentially damaging effects following acute, neuronal injuries.

A common feature of AD senile plaques is the presence of activated astrocytes, characterized by increased glial fibrillary acidic protein and bFGF content *(80)*. IL-1β, secreted by activated microglia as described above, can induce a reactive phenotype in astroglial cells *(69)*. Whereas astrocytes are relatively resistant to Aβ in terms of toxicity, Aβ was shown to contribute directly to the activation of the astrocytes in culture *(70)*. Aβ might then indirectly increase the expression of growth factors like bFGF. Interestingly, bFGF was previously found to be present in plaques *(81)* and protects hippocampal neurons in culture against Aβ neurotoxicity *(82)*. This activation may therefore have positive effects, to a certain extent, on degenerating neurons. Interestingly, the reactive gliosis induced by Aβ seems to be region-specific and the cultured astrocytes from vulnerable brain regions in AD (i.e., hippocampus, cerebral cortex) are more sensitive to Aβ than the astrocytes derived from less vulnerable regions in AD, like cerebellum or spinal cord *(83)*.

Astrocytes serve an important function in the regulation of extracellular glutamate concentration. They express high levels of specific glutamate transporters. Previous studies have reported a sensitivity of the glutamate transporters to free radicals *(84)*. Since Aβ increases lipid peroxidation and ROS in neurons (*see* Fig. 1), one can expect an impairment of glial glutamate transporters by Aβ. A recent study by Harris et al. *(20)* indicates this is indeed the case. A decrease in glutamate uptake by glial cells is likely to enhance the synaptic concentration of glutamate and to promote excitotoxicity.

Aβ and Lymphocytes

Circulating lymphocytes have been shown to be affected by Aβ. The mitogen-induced Ca^{2+} rise was potentiated by Aβ in human lymphocytes from young donors *(85)*. Furthermore, this mitogen-induced activation of leukocytes was previously shown to enhance the secretion of sAPP *(86)*. The potentiating effect of Aβ on the mitogen-elicited Ca^{2+} response may be mediated by a decrease in the fluidity of lymphocyte plasma membrane *(87)*. Interestingly, the sensitivity of lymphocytes from AD patients to the potentiating effect of Aβ on the mitogen-induced Ca^{2+} rise was reduced *(88)*. Whereas the role of this Aβ effect in the pathogenesis of AD is not well understood, the decreased sensitivity of lymphocytes

to Aβ in AD may represent an interesting peripheral marker of the disease.

Conclusions and Possible Therapeutic Interventions

The recent findings concerning the mechanisms of Aβ production and cytotoxicity described above suggest a number of potential intervention points in the pathological progression of AD. These include inhibition of Aβ production by inhibiting β-secretase cleavage, enhancement of Aβ-clearance pathways, and blocking, at the cellular level, the toxicity of aggregated Aβ. Although there are currently no clinical treatments that address the first two approaches, there are a growing number of candidates for the third. As the mechanisms of toxicity of Aβ continue to be elucidated, more and better protective agents will be identified. As we have shown in this article, antioxidants have so far proven, in vitro, to be the most effective agents for blocking all reported actions of Aβ. These actions range from the aggregation of Aβ, to its induction of ROS, to its impairment of function of cellular proteins, to its neurotoxicity. Another potential treatment uses anticonvulsants and K^+ channel agonists (Fig. 3). By keeping the cells hyperpolarized, the compounds are able to overcome the depolarizing effect of Aβ-induced impairment of the plasma membrane ion-motive pumps and aid in protecting the cells from excitotoxicity. Many of these compounds have already been approved for use in other clinical conditions and are therefore poised for rapid movement to clinical trials for AD.

Neurotrophic factors make up a third class of potential treatment compounds. Numerous neurotrophic factors have been shown to protect cultured neurons against Aβ toxicity. Delivery of growth factors into the brain is difficult. As the mechanisms by which growth factors protect are identified, activation of pathways downstream from the receptor/ligand binding will yield more "clinically friendly" treatments. For example, staurosporine and K252a can activate receptor tyrosine kinase signaling cascades and protect rat hippocampal neurons from oxidative and excitotoxic insults *(89)*. Ceramide is another example (Fig. 4). Ceramide is known to activate the transcription factor NFκB and has been shown to protect cultured hippocampal neurons against Aβ toxicity *(90)*. Because much of the available data concerning mechanisms of amyloid toxicity and oxidative injury to cells are on cell-culture data, it is imperative that better animal models be developed in order to test in vivo the agents that have proven so protective in vitro.

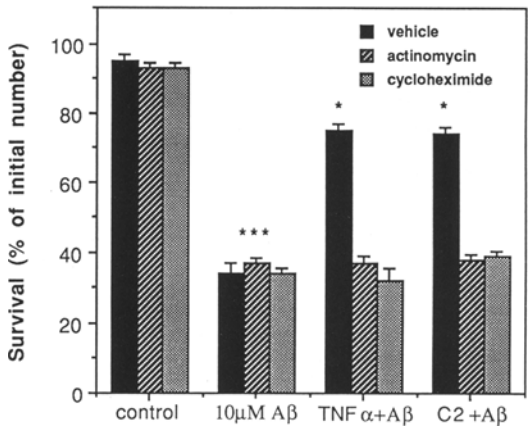

Fig. 4. TNFα and ceramide protect hippocampal neurons from Aβ toxicity by a mechanism that involves transcription and translation. Cultures were pretreated for 16 h with TNFα or C2-ceramide in the presence or absence of actinomycin D (5 μ*M*) or cycloheximide (1 μ*M*). Cultures were then treated with Aβ (10 μ*M*) for 24 h and neuronal survival was determined. $*p < 0.01$ as compared to Aβ-treated cultures not exposed to TNFα or C2; $***p < 0.001$ as compared to corresponding control values. ANOVA with Scheffe's *posthoc* test.

Acknowledgments

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