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Perturbed endoplasmic reticulum function, synaptic apoptosis and the pathogenesis of Alzheimer's disease

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Abstract

Endoplasmic reticulum (ER) appears to be a focal point for alterations that result in neuronal dysfunction and death in Alzheimer's disease (AD). Aberrant proteolytic processing and/or trafficking of the β-amyloid precursor protein (APP) in ER may promote neuronal degeneration by increasing the levels of the neurotoxic forms of β-amyloid (Aβ) and by decreasing the levels of the neuroprotective secreted form of APP (sAPPα).

Some cases of AD are caused by mutations in the genes encoding presenilin 1 (PS1). When expressed in cultured neuronal cells and transgenic mice, PS1 mutations cause abnormalities in ER calcium homoeostasis, enhancing the calcium responses to stimuli that activate IP_3- and ryanodine-sensitive ER calcium pools. Two major consequences of this disrupted ER calcium regulation are altered proteolytic processing of APP and increased vulnerability of neurons to apoptosis and excitotoxicity. The impact of PS1 mutations and aberrant APP processing is particularly great in synaptic terminals. Perturbed synaptic calcium homoeostasis promotes activation of apoptotic cascades involving production of Par-4 (prostate apoptosis response-4), mitochondrial dysfunction and caspase activation. Aβ42 (the 42-amino-acid form of Aβ) induces membrane lipid peroxidation in synapses and dendrites resulting in impairment of membrane ion-motive ATPases and glucose and glutamate transporters. This disrupts synaptic ion and energy homoeostasis thereby promoting synaptic degeneration. In contrast, sAPPα activates signalling pathways that protect

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synapses against excitotoxicity and apoptosis. In the more common sporadic forms of AD, the initiating causes of the neurodegenerative cascade are less well defined, but probably involve increased levels of oxidative stress and impaired energy metabolism. Such alterations have been shown to disrupt neuronal calcium homoeostasis in experimental models, and may therefore feed into the same neurodegenerative cascade initiated by mutations in presenilins and APP. Perturbed synaptic ER calcium homoeostasis and consequent alterations in APP processing appear to be pivotal events in both sporadic and familial forms of AD.

Introduction

Alzheimer's disease (AD) is characterized by degeneration of synapses, neuronal death and associated deposition of β-amyloid (Aβ). Prior to the identification of AD-linked mutations in APP and presenilins, considerable evidence had accumulated that supported important roles for perturbed neuronal calcium homoeostasis and increased levels of oxidative stress in the pathogenesis of AD [1–5]. It also became evident that degeneration of synapses was strongly correlated with dementia in AD patients [6,7]. The identification of mutations in APP and the presenilins which cause early-onset inherited forms of AD has dramatically accelerated the process of determining the specific sequence of events that results in neuronal degeneration in AD. Studies of cultured cell lines and transgenic mice expressing APP and PS1 mutations have begun to delineate the molecular, biochemical and cell biological underpinnings of the neurodegenerative process in AD. This chapter describes recent findings originating from such studies, focusing on the alterations in endoplasmic reticulum (ER) function that appear to be the pivotal events that disrupt synaptic function and promote synapse degeneration and ultimately neuronal death in AD.

How do β-amyloid precursor protein (APP) mutations promote synaptic dysfunction and degeneration?

The gene encoding APP was the first to be identified as a locus for mutations that cause autosomal-dominant inherited AD [8]. Several different mutations have been identified. One mutation involves a single amino acid substitution located just N-terminal to the Aβ sequence, another mutation involves a two-residue change located just C-terminal to the Aβ sequence, and a third mutation involves a single amino acid change within the Aβ sequence. One consequence of these APP mutations is that they result in increased production of Aβ, particularly Aβ42 (the 42-amino-acid form of Aβ). Since Aβ has been shown to be neurotoxic, and to increase neuronal vulnerability to apoptosis and excitotoxicity, it has been proposed that the increase in Aβ production is the key alteration that leads to amyloid deposition and neuronal degeneration in patients with APP mutations [9]. The mechanism whereby Aβ damages neurons involves induction of membrane lipid peroxidation, which

results in impairment of membrane ion-motive ATPases and glucose and glutamate transporters. This, in turn, promotes membrane depolarization and cellular energy depletion, and thereby renders neurons vulnerable to excitotoxicity and apoptosis [3,5,10–12].

APP is synthesized and glycosylated in the ER and is then 'sorted' to the plasma membrane, where it forms an integral membrane protein with one membrane-spanning domain [9]. An intriguing feature of APP is that it is enzymically cleaved in response to neuronal activity (depolarization and activation of receptors is linked to phospholipase C activation) by a yet-to-be identified enzyme activity called α-secretase. The cleavage releases a large N-terminal domain, called sAPPα, from the cell surface. sAPPα plays important roles in modulating neuronal excitability, and in developmental and synaptic plasticity as indicated by the following findings. Exposure of cultured embryonic hippocampal neurons to sAPPα results in activation of high-conductance, charybdotoxin-sensitive, potassium channels resulting in membrane hyperpolarization and reduced calcium influx through voltage-dependent calcium channels and ionotropic glutamate receptor channels [13,14]. Dendrite outgrowth in developing hippocampal neurons is increased following exposure to sAPPα [15]. Treatment of hippocampal slices from adult rats with sAPPα results in a shift in the frequency dependence of long-term depression of synaptic transmission, and an enhancement of long-term potentiation of synaptic transmission [16]. We have proposed that APP mutations promote neuronal degeneration by decreasing the levels of sAPPα [13,14,17] and, indeed, studies have documented that APP mutations do decrease production of sAPPα and that levels of sAPPα are decreased in the cerebrospinal fluid of AD patients [18,19].

Both Aβ and sAPPα exert direct effects on synaptic terminals. Such local effects of these APP derivatives are likely to play important roles in the early stages of AD (Figure 1). Studies of synaptosomes have shown that Aβ is able to induce membrane lipid peroxidation, and impair the function of ion, glucose and glutamate transporters in synaptic terminals [5,20]. These adverse effects of Aβ can be reduced by treating synaptosomes with antioxidants such as vitamin E and oestrogens [5,20,21]. Interestingly, Aβ and oxidative insults can induce local activation of apoptotic cascades involving prostate apoptosis response-4 (Par-4), caspases and mitochondrial dysfunction in dendrites and synaptic terminals [22–24]. The latter findings suggest that neuronal apoptosis in AD may be initiated by events occurring in synapses. Reduced levels of sAPPα may also promote synapse degeneration, since treatment of cultured hippocampal neurons with sAPPα decreases their vulnerability to synaptically driven excitotoxicity [13]. sAPPα can also protect synaptosomes against the damaging effects of exposure to Aβ and iron [25], as in intact neurons, the synaptoprotective effects of sAPPα involve activation of a signalling pathway that employs cyclic GMP as a second messenger [14,25–27].

Finally, several observations suggest roles for alterations in ER function in the pathogenic actions of APP mutations. It has been proposed that APP mutations result in increased Aβ production because they alter trafficking of APP such that it is exposed to different enzymic environments [28–31]. Indeed,

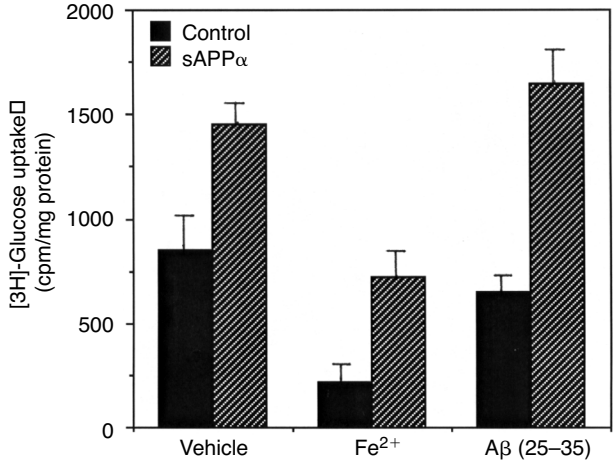

Figure 1 Direct actions of Aβ and sAPPα on synaptic terminals. Rat cortical synaptosomes were pretreated with sAPPα or saline (control). Synaptosomes were then exposed to either vehicle, Fe^{2+} or Aβ25–35 for 4 hours and the levels of radiolabelled glucose uptake were quantified. Values are means ± S.E.M. of determinations made in 4–6 synaptosome samples. Reproduced from Mattson, M.P., Cuo, Z.H. and Geiger, J.D. Secreted form of amyloid precursor protein enhances basal glucose and glutamate transport and protects against oxidative impairment of glucose and glutamate transport in synaptosomes by a cyclic GMP-mediated mechanism. (1999) J. Neurochem. **73 (2)**, 532–537, with permission. © (1999) Lippincott, Williams & Wilkins.

treatment of cells with agents that disrupt protein trafficking in the ER results in increased Aβ production. We recently provided evidence that perturbed ER calcium homoeostasis may contribute to the neurodegenerative effects of altered APP processing. Thus, agents such as dantrolene and xestospongin that block calcium release from ER can protect neurons against Aβ toxicity (Figure 2) [32,33]. In addition, treatment of cultured hippocampal neurons with sAPPα increases their resistance to apoptosis induced by thapsigargin, an agent that kills cells by inducing massive release of calcium from ER (Figure 2). On the other hand, metabolic stress and increased intracellular calcium levels alter APP processing such that levels of Aβ are increased and levels of sAPPα decreased [34,35]. Collectively, the data suggest that the neurodegenerative cascade in AD involves a feed-forward process in which metabolic compromise and perturbed calcium homoeostasis alter APP processing, inducing oxidative and metabolic stress and increasing levels of intracellular calcium (Figure 3).

How do presenilin mutations promote synaptic dysfunction and degeneration?

Several different laboratories have reported perturbed ER calcium regulation [32,33,36,37] and altered APP processing, resulting in increased production of Aβ42 and decreased production of sAPPα [38–40], as two consequences of expressing PS1 mutations in cultured cells. PC12

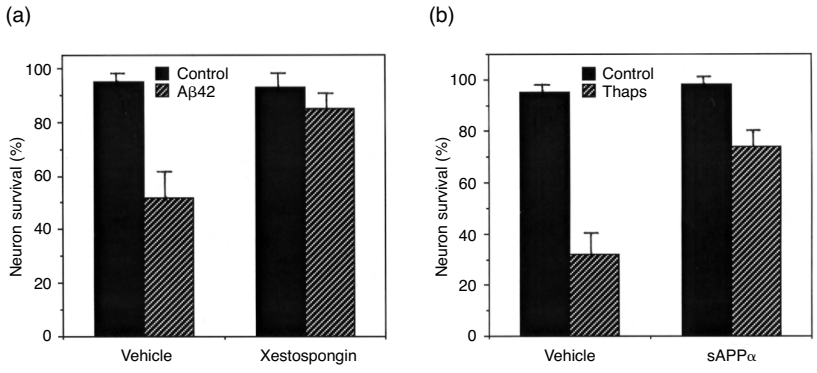

Figure 2 Evidence for the differential effects of Aβ and sAPPα on calcium release from ER and their roles in modifying neuronal death. Rat hippocampal cultures were pretreated for 2 hours with either 1 μM xestospongin (a) or 1 nM sAPPα (b). Cultures were then exposed to either 10 μM Aβ42 (a) or 1 μM thapsigargin (Thaps) (b) for 24 hours. Neuronal survival was quantified and values are means ± S.E.M. of determinations made in four separate cultures.

(pheochromocytoma) cells overexpressing PS1 mutations (Leu^{286}→Val and Met^{146}→Val) showed a significantly greater increase in levels of intracellular calcium, in response to activation of receptors linked to calcium release from

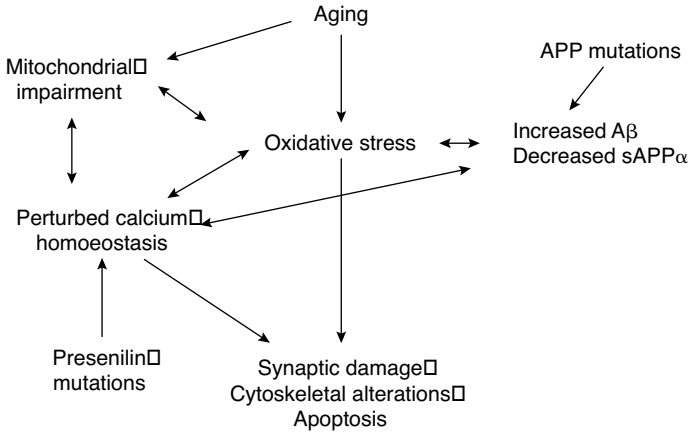

Figure 3 Inter-related mechanisms involved in the pathogenesis of sporadic and familial AD. In sporadic forms of AD, age-related increases in cellular oxidative stress and metabolic compromise result in altered proteolytic processing of APP, disruption of cellular calcium homoeostasis and mitochondrial dysfunction. In the case of presenilin mutations, the primary alteration appears to be perturbed ER calcium regulation which, in turn, results in altered APP processing and oxidative stress. Increased levels of oxidative stress and disruption of calcium homoeostasis result in neuronal apoptosis and excitotoxicity.

IP_3-sensitive stores, compared to untransfected PC12 cells and PC12 cells overexpressing wild-type PS1 ([32]; and M.P. Mattson, unpublished work]. PC12 cells overexpressing mutant PS1 exhibited increased vulnerability to apoptosis induced by trophic factor withdrawal and exposure to Aβ. This endangering action of the PS1 mutations could be counteracted by treating cells with agents that block calcium release from IP_3- and ryanodine-sensitive stores [32,33]. Thus, calcium release from ER is a necessary step in the pro-apoptotic actions of mutant PS1. Additional studies showed that overexpression of the calcium-binding protein calbindin [36] and treatment of cells with antioxidants [33] can counteract the pro-apoptotic action of mutant PS1, indicating that increased levels of cytoplasmic calcium and oxidative stress make important contributions to the cell death process in such cell culture models.

Cells expressing mutant PS1 exhibit an abnormal pattern of activation of the anti-apoptotic transcription factor nuclear factor κB (NF-κB) following exposure to oxidative insults including Aβ [41]. Treatment with sAPPα protected the cells against the pro-apoptotic actions of mutant PS1 and normalized the pattern of NF-κB activation. PC12 cells overexpressing mutant PS1 exhibit enhanced production of the pro-apoptotic protein Par-4 following trophic factor withdrawal and exposure to Aβ [42]. The increased Par-4 production may contribute to reduced NF-κB activation [43].

More recently we showed that hippocampal neurons from PS1 mutant knockin mice are more vulnerable to excitotoxicity [44] and apoptosis [45] than are hippocampal neurons from wild-type mice. Calcium-imaging studies in hippocampal cultures from these mice showed that neurons expressing mutant PS1 exhibit enhanced calcium responses to glutamate when compared with wild-type neurons [44].

Presenilin mutations can cause local disruption of calcium homoeostasis and dysfunction in synapses. Synaptosomes prepared from the cerebral cortex of PS1 mutant transgenic mice exhibit enhanced elevations of intracellular calcium levels in response to membrane depolarization and exposure to Aβ, compared to synaptosomes from non-transgenic mice and mice overexpressing wild-type PS1 [46]. The magnitude of mitochondrial dysfunction and caspase activation induced by Aβ and a metabolic insult were also enhanced in synaptosomes from PS1 mutant mice. Treatment of synaptosomes with the calcium chelator BAPTA-AM [(bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid tetrakis (acetoxymethyl ester)] and dantrolene counteracted the adverse effects of the PS1 mutation on synaptic mitochondrial function, indicating that calcium release from ER was central to the adverse effects of PS1 mutations in synaptic terminals. More recently, Parent and colleagues [47] have demonstrated alterations in synaptic function in hippocampal slices from PS1 mutant transgenic mice. Collectively, the data support a key role for abnormal synaptic calcium homoeostasis and mitochondrial dysfunction in the pathogenic mechanism of PS1 mutations.

What is the specific mechanism whereby PS1 mutations disrupt ER calcium regulation? One possibility is that PS1 directly interacts with one or more calcium-regulating proteins and that PS1 mutations alter such interactions. Several candidates for such PS1-interacting proteins have recently been identi-

fied. Calsenilin is a cytoplasmic calcium-binding protein recently shown to interact with PS1 in the yeast two-hybrid assay [48]. Additional data in the latter study suggest that the calsenilin–PS1 interaction can alter enzymic cleavage of PS1. Also using the yeast two-hybrid approach, Kim and colleagues [49] reported that PS2 interacts with sorcin, a protein known to modulate the function of ryanodine receptors. However, PS1 did not interact with sorcin suggesting that this interaction may not play a role in the effects of presenilin mutations on ER calcium homoeostasis. Using a co-immunoprecipitation approach we have recently found that PS1 interacts with a ryanodine receptor complex in cultured neurons and mouse neocortical tissue [49a]. PS1 mutations might have indirect effects on ER calcium homoeostasis. For example, we recently found that levels of expression of the type 3 ryanodine receptor are greatly increased in PC12 cells overexpressing mutant PS1 [49a].

Role of the novel apoptotic protein Par-4 in synaptic apoptosis and AD

Par-4 is a 38 kDa protein identified by differential screening of prostate tumour cells for genes up-regulated during apoptosis [50]. Par-4 mRNA and protein levels were found to be increased in post-mortem brain tissue samples of hippocampus and inferior parietal cortex from AD patients compared to samples from age-matched control patients [42]. The latter study also showed that many neurofibrillary tangle-bearing neurons exhibit high levels of Par-4, suggesting that it increases specifically in neurons that degenerate. Experimental studies have shown that Par-4 protein levels increase rapidly in PC12 cells and primary hippocampal neurons in response to various apoptotic insults including trophic factor withdrawal and exposure to Aβ [42]. Suppression of the insult-induced increase in Par-4 levels, using antisense technology, prevents neuronal apoptosis, demonstrating a requirement for Par-4 in the cell death process [42]. Oxidative stress and calcium influx are triggers for Par-4 induction, and antioxidants and manipulations that reduce levels of intracellular calcium can suppress Par-4 expression [42,51]. Par-4 production occurs prior to, and is required for, mitochondrial dysfunction and caspase activation [42].

Par-4 contains both a leucine-zipper domain and a partially overlapping death domain near its C-terminus [50]. The leucine-zipper domain of Par-4 is required for its pro-apoptotic action since overexpression of Par-4 lacking the leucine-zipper domain does not promote apoptosis, and overexpression of the leucine-zipper domain alone acts in a dominant-negative manner to prevent apoptosis [42]. Several proteins have been identified that can interact with Par-4 and may mediate its pro-apoptotic action. Initial studies of tumour cells identified protein kinase Cζ (PKCζ) [52] as one protein that interacts with Par-4; the interaction inhibits the kinase activity of the enzyme. Data showing that activation of PKCζ can prevent apoptosis [53] suggest a role for the latter interaction in promoting apoptosis. We have found that Par-4 interacts with PKCζ in embryonic hippocampal neurons (S.L. Chan and M.P. Mattson, unpublished work). Interestingly, we also recently discovered that Par-4 suppresses activa-

tion of the anti-apoptotic transcription factor NF-κB [43]. Since PKCζ may mediate activation of NF-κB in cells exposed to potentially lethal insults, suppression of the PKCζ–NF-κB pathway may play an important role in the pro-apoptotic action of Par-4 (Figure 4). NF-κB exists in the cytoplasm of neurons in an inactive three-subunit complex that includes the transcription factor dimer (typically p50/p65 heterodimers) and an inhibitory subunit called I-κBα. NF-κB is activated when signals such as cytokines, calcium and oxidative stress induce phosphorylation of I-κB. This results in dissociation of the p50/p65 dimer which then translocates to the nucleus and binds to specific sequences in the enhancer region of target genes such as Mn-superoxide dismutase, calbindin and inhibitor of apoptosis (IAP) proteins [54].

Par-4 may play a particularly important role in post-synaptic terminals and dendrites. Exposure of synaptosomes and primary rat hippocampal cell cultures to apoptotic insults resulted in relatively rapid increases (1–2 hours) in Par-4 protein levels in dendrites and synaptic terminals [55]. Treatment of syn-

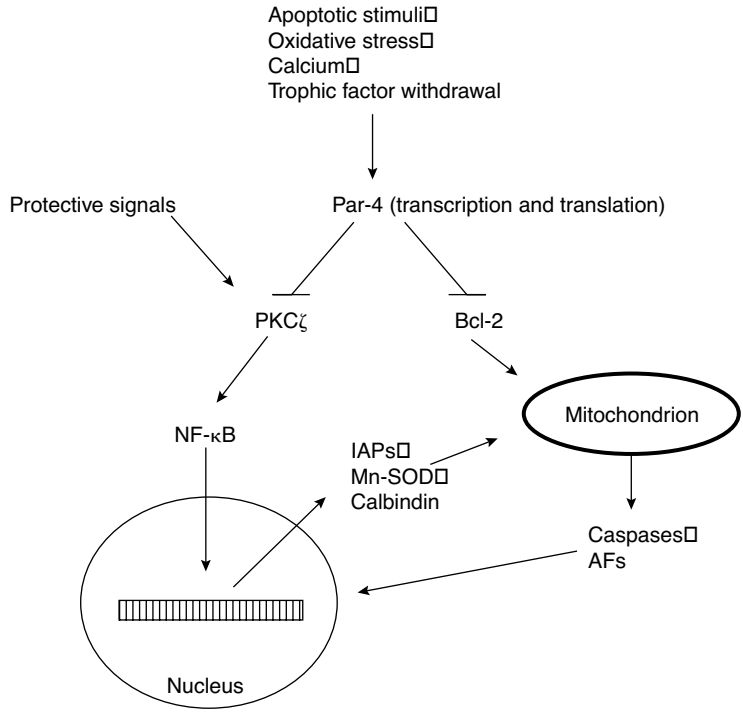

Figure 4 Working model of the mechanisms whereby Par-4 promotes neuronal apoptosis. Par-4 protein levels are rapidly increased in neurons following exposure to various apoptotic and excitotoxic insults. Available data suggest that Par-4 can suppress activation of the anti-apoptotic transcription factor NF-κB, apparently by suppressing activity of PKCζ, a kinase that can induce activation of NF-κB. In addition, Par-4 interacts with Bcl-2, and this interaction may lead to mitochondrial dysfunction and caspase activation. AF, apoptotic factor; IAP, inhibitor of apoptosis protein.

aptosomes with cycloheximide prevented the increases in Par-4 levels in synaptosomes following exposure to apoptotic insults, indicating that protein synthesis was required for Par-4 induction. These intriguing findings open a new avenue of investigation in the neuronal apoptosis field because they provide direct evidence that expression of a death-related protein can be induced locally in post-synaptic terminals. Moreover, Par-4 induction by apoptotic insults in synaptic terminals can be suppressed using antisense technology, and such suppression of Par-4 expression results in a marked attenuation of mitochondrial dysfunction and caspase activation [55]. Thus, Par-4 appears to play a central role in synaptic apoptotic cascades.

What about sporadic AD?

While studies of the pathogenic actions of APP and presenilin mutations have been invaluable in deciphering the molecular and cellular mechanisms underlying AD, the neurodegenerative process in the more common sporadic forms of AD remains unknown. The available data suggest the following scenario: subtle genetic factors, aging and the environment (e.g. diet, lifestyle, exposure to toxins or trauma) interact to increase levels of oxidative and metabolic stress in neurons. The latter statement is supported by compelling data demonstrating increased levels of oxyradical-mediated damage to proteins, lipids and DNA [56], and reduced glucose availability to cells [57] in the brain, during normal aging and more so in AD. Increased oxidative stress and reduced energy availability promote disruption of neuronal calcium homoeostasis. Perturbed calcium homoeostasis results in altered proteolytic processing of APP, which in turn leads to increased production of Aβ and decreased production of sAPPα. A vicious cycle is thus initiated that promotes neuronal apoptosis and excitotoxicity.

Genetic factors that may increase risk for sporadic AD are being identified. One such factor is *APOE* (apolipoprotein) genotype [58]; ApoE4 increases the risk, while ApoE2 decreases it. ApoEs appear to exert neurotrophic/neuroprotective actions, with ApoE2 and ApoE3 being more effective than ApoE4. We have recently found that ApoE2 protects neurons against oxidative injury by a mechanism that may involve binding of the toxic aldehyde product of lipid peroxidation, 4-hydroxynonenal [59]. The latter findings are intriguing because the differences between the three isoforms lie in cysteine residues at two positions (ApoE4 lacks cysteines at those residues, while ApoE3 and ApoE2 contain 1 and 2 cysteines respectively). 4-Hydroxynonenal covalently modifies proteins on cysteine residues. Thus, ApoE2 and ApoE3 isoforms may act to detoxify 4-hydroxynonenal under conditions of oxidative stress.

Environmental factors that may increase risk for AD include high calorie intake [60–62], history of head injury [63], low intake of antioxidants [64] and low level of mental challenges [65]. Reduced calorie intake may reduce the risk of developing AD by decreasing free radical production (a mechanism thought to underlie increased lifespan in animals maintained on a food restriction) and/or by enhancing cellular resistance to oxidative and metabolic insults

[66,67]. Living an intellectually enriched lifestyle may increase resistance of neurons to age-related degeneration by increasing production of neurotrophic factors and stress proteins [68]. What seems to be clear is that genetic and environmental factors converge on common neurodegenerative pathways involving oxidative stress, impaired energy metabolism and perturbed cellular calcium homoeostasis.

Implications for preventative and therapeutic approaches to AD

The following potentially beneficial preventative and therapeutic strategies are suggested by the kinds of data described above.

- Reduced calorie intake – this lifestyle approach may increase resistance of neurons to several age-related disorders including AD, Parkinson's disease, Huntington's disease and stroke [60,66,69]. This approach may be effective in both sporadic and familial forms of AD [61].
- Increased antioxidant intake – oxidative stress appears to make a major contribution to the neurodegenerative process in both sporadic and familial AD. Particularly valuable may be antioxidants that remove mitochondrial reactive oxygen species and suppress membrane lipid peroxidation.
- Drugs that stabilize neuronal calcium homoeostasis. Prototypical examples include dantrolene and xestospongin to suppress calcium release from ER, and calcium channel antagonists to suppress calcium influx through plasma membrane channels.
- Mental calisthenics – use of neuronal circuits increases their resistance to adversity. This may occur as a result of the up-regulation of neurotrophic factors and certain stress proteins [68].

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References

1. Mattson, M.P. (1990) *Neuron* **4**, 105–117
2. Nixon, R.A., Saito, K.I., Grynspan, F., Griffin, W.R., Katayama, S., Honda, T., Mohan, P.S., Shea, T.B. and Beerman, M. (1994) *Ann. N.Y. Acad. Sci.* **747**, 77–91
3. Mattson, M.P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I. and Rydel, R.E. (1992) *J. Neurosci.* **12**, 376–389
4. Goodman, Y. and Mattson, M.P. (1994) *Exp. Neurol.* **128**, 1–12
5. Mark, R.J., Hensley, K., Butterfield, D.A. and Mattson, M.P. (1995) *J. Neurosci.* **15**, 6239–6249
6. Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A. and Katzman, R. (1991) *Ann. Neurol.* **30**, 572–580
7. DeKosky, S.T., Scheff, S.W. and Styren, S.D. (1996) *Neurodegeneration* **5**, 417–421
8. Selkoe, D.J. (1993) *Trends Neurosci.* **16**, 403–409
9. Mattson, M.P. (1997) *Physiol. Rev.* **77**, 1081–1132
10. Mark, R.J., Lovell, M.A., Markesbery, W.R., Uchida, K. and Mattson, M.P. (1997) *J. Neurochem.* **68**, 255–264

11. Mark, R.J., Pang, Z., Geddes, J.W., Uchida, K. and Mattson, M.P. (1997) *J. Neurosci.* **17**, 1046–1054
12. Blanc, E.M., Keller, J.N., Fernandez, S. and Mattson, M.P. (1998) *Glia* **22**, 149–160
13. Mattson, M.P., Cheng, B., Culwell, A.R., Esch, F., Lieberburg, I. and Rydel, R.E. (1993) *Neuron* **10**, 243–254
14. Furukawa, K., Barger, S.W., Blalock, E. and Mattson, M.P. (1996) *Nature (London)* **379**, 74–78
15. Mattson, M.P. (1994) *J. Neurobiol.* **25**, 439–450
16. Ishida, A., Furukawa, K., Keller, J.N. and Mattson, M.P. (1997) *NeuroReport* **8**, 2133–2137
17. Smith-Swintosky, V., Pettigrew, L., Craddock, S., Culwell, A., Rydel, R. and Mattson, M.P. (1994) *J. Neurochem.* **63**, 781–784
18. Palmert, M.R., Usiak, M., Mayeux, B., Raskind, M., Tourtellotte, W.W. and Younkin, S.G. (1990) *Neurology* **40**, 1028–1034
19. Lannfelt, L., Basun, H., Wahlund, L.O., Rowe, B.A. and Wagner, S.L. (1995) *Nat. Med. (N.Y.)* **1**, 829–832
20. Keller, J.N., Pang, Z., Geddes, J.W., Begley, J.G., Germeyer, A., Waeg, G. and Mattson, M.P. (1997) *J. Neurochem.* **69**, 273–284
21. Keller, J.N. and Mattson, M.P. (1997) *J. Neurosci. Res.* **50**, 522–530
22. Mattson, M.P., Keller, J.N. and Begley, J.G. (1998). *Exp. Neurol.* **153**, 35–48
23. Mattson, M.P., Partin, J. and Begley, J.G. (1998) *Brain Res.* **807**, 167–176
24. Duan, W., Rangnekar, V. and Mattson, M.P. (1999) *J. Neurochem.* **72**, 2312–2322
25. Mattson, M.P., Guo, Z.H. and Geiger, J.D. (1999) *J. Neurochem.* **73**, 532–537
26. Barger, S.W., Fiscus, R.R., Ruth, P., Hofmann, F. and Mattson, M.P. (1995) *J. Neurochem.* **64**, 2087–2096
27. Furukawa, K., Sopher, B., Rydel, R.E., Begley, J.G., Martin, G.M. and Mattson, M.P. (1996) *J. Neurochem.* **67**, 1882–1896
28. Cai, X., Golde, T. and Youkin, S. (1993) *Science* **259**, 514–516
29. Citron, M., Vigo-Pelfrey, C., Teplow, D.B., Miller, C., Schenk, D., Johnston, J., Winblad, B., Venizelos, N., Lannfelt, L. and Selkoe, D.J. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11993–11997
30. Haass, C., Hung, A., Selkoe, D. and Teplow, D. (1994) *J. Biol. Chem.* **269**, 17741–17748
31. Haass, C., Lemere, C.A., Capell, A., Citron, M., Seubert, P., Schenk, D. and Selkoe, D. (1995) *Nat. Med. (N.Y.)* **1**, 1291–1296
32. Guo, Q., Furukawa, K., Sopher, B.L., Pham, D.G., Robinson, N., Martin, G.M. and Mattson, M.P. (1996) *NeuroReport* **8**, 379–383
33. Guo, Q., Sopher, B.L., Pham, D.G., Furukawa, K., Robinson, N., Martin, G.M. and Mattson, M.P. (1997) *J. Neurosci.* **17**, 4212–4222
34. Gabuzda, D., Busciglio, J., Chen, L.B., Matsudaira, P. and Yankner, B.A. (1994) *J. Biol. Chem.* **269**, 13623–13628
35. Querfurth, H.W. and Selkoe, D.J. (1994) *Biochemistry* **33**, 4550–4561
36. Guo, Q., Christakos, S., Robinson, N. and Mattson, M.P. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3227–3232
37. Leissring, M.A., Paul, B.A., Parker, I. and Cotman, C.W. and LaFerla, F.M. (1999) *J. Neurochem.* **72**, 1061–1068
38. Scheuner, D. Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., et al. (1996) *Nat. Med. (N.Y.)* **2**, 864–870
39. Guo, Q., Sebastian, L., Sopher, B.L., Miller, M.W., Glazner, G.W., Ware, C.B., Martin, G.M. and Mattson, M.P. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4125–4130
40. Ancolio, K., Marambaud, P., Dauch, P. and Checler, F. (1997) *J. Neurochem.* **69**, 2494–2499
41. Guo, Q., Robinson, N. and Mattson, M. P. (1998) *J. Biol. Chem.* **273**, 12341–12351
42. Guo, Q., Fu, W., Xie, J., Luo, H., Sells, S.F., Geddes, J.W., Bondada, V., Rangnekar, V.M. and Mattson, M.P. (1998) *Nat. Med. (N.Y.)* **4**, 957–962

43. Camandola, S. and Mattson, M.P. (2000) *J. Neurosci. Res.* **61**, 134–139
44. Guo, Q., Fu, W., Sopher, B.L., Miller, M.W., Ware, C.B., Martin, G.M. and Mattson, M.P. (1999) *Nat. Med. (N.Y.)* **5**, 101–107
45. Guo, Q., Sebastian, L., Sopher, B.L., Miller, M.W., Ware, C.B., Martin, G.M. and Mattson, M.P. (1999) *J. Neurochem.* **72**, 1019–1029
46. Begley, J.G., Duan, W., Duff, K. and Mattson, M.P. (1999) *J. Neurochem.* **72**, 1030–1039
47. Parent, A., Linden, D.J., Sisodia, S.S. and Borchelt, D.R. (1999) *Neurobiol. Dis.* **6**, 56–62
48. Buxbaum, J.D., Choi, E.K., Luo, Y., Lilliehook, C., Crowley, A.C., Merriam, D.E. and Wasco, W. (1998) *Nat. Med. (N.Y.)* **4**, 1177–1181
49. Kim, T.W., Pettingell, W.P. and Tanzi, R.E. (1998) *Soc. Neurosci. Abstr.* **34**, 757
49a. Chan, S.L., Mayne, M., Holden, C.P., Geiger, J.D. and Mattson, M.P. (2000) *J. Biol. Chem.* **275**, 18195–18200
50. Sells, S.F., Han, S.S., Muthukkumar, S., Maddiwar, N., Johnstone, R., Boghaert, E., Gillis, D., Liu, G., Nair, P., Monnig, S., et al. (1997) *Mol. Cell. Biol.* **17**, 3823–3832
51. Chan, S.L., Tammariello, S.P., Estus, S. and Mattson, M.P. (1999) *J. Neurochem.* **73**, 502–512
52. Diaz-Meco, M.T., Municio, M.M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L. and Moscat, J. (1996) *Cell* **86**, 777–786
53. Berra, E., Municio, M.M., Sans, L., Frutos, S., Diaz-Meco, M.T. and Moscat, J. (1997) *Mol. Cell. Biol.* **17**, 4346–4354
54. Mattson, M.P., Culmsee, C., Yu, Z. and Camandola, S. (2000) *J. Neurochem.* **74**, 443–456
55. Duan, W., Rangnekar, V. and Mattson, M.P. (1999) *J. Neurochem.* **72**, 2312–2322
56. Markesbery, W.R. (1997) *Free Radic. Biol. Med.* **23**, 134–147
57. Mattson, M.P., Pedersen, W.A., Duan, W. and Camandola, S. (1999) *N.Y. Acad. Sci.* **893**, 154–175
58. Saunders, A.M., Strittmatter, W.J., Schmechel, D., George-Hyslop, P.H., Pericak-Vance, M.A., Joo, S.H., Rosi, B.L., Gusella, J.F., Crapper-MacLachlan, D.R., Alberts, M.J., et al. (1993) *Neurology* **43**, 1467–1472
59. Pedersen, W.A., Chan, S.L. and Mattson, M.P. (2000) *J. Neurochem.* **74**, 1426–1433
60. Bruce-Keller, A.J., Umberger, G., McFall, R. and Mattson, M.P. (1999) *Ann. Neurol.* **45**, 8–15
61. Zhu, H., Guo, Q. and Mattson, M.P. (1999) *Brain Res.* **842**, 224–229
62. Mayeux, R., Costa, R., Bell, K., Merchant, C., Tang, M.X. and Jacobs, D. (1999) *Neurology* **59**, S296–S297
63. Mayeux, R., Ottman, R., Maestre, G., Ngai, C., Tang, M.X., Ginsberg, H., Chun, M., Tycko, B. and Shelanski, M. (1995) *Neurology* **45**, 555–557
64. Morris, M.C., Beckett, L.A., Scherr, P.A., Hebert, L.E., Bennett, D.A., Field, T.S. and Evans, D.A. (1998) *Alzheimer Dis. Assoc. Disord.* **12**, 121–126
65. Stern, Y., Gurland, B., Tatemichi, T.K., Tang, M.X., Wilder, D. and Mayeux, R. (1994) *J. Am. Med. Assoc.* **271**, 1004–1010
66. Duan, W. and Mattson, M.P. (1999) *J. Neurosci. Res.* **57**, 195–206
67. Lee, J., Bruce-Keller, A.J., Kruman, Y., Chan, S.L. and Mattson, M.P. (1999) *J. Neurosci. Res.* **57**, 48–61
68. Mattson, M.P. and Lindvall, O. (1997) in *The Aging Brain* (Mattson, M.P. and Geddes, J.W., eds), pp. 299–345, JAI Press, Greenwich, CT
69. Yu, Z.F. and Mattson, M.P. (1999) *J. Neurosci. Res.* **57**, 830–839