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Brain Oxidative Stress and Rescue Mechanisms

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The amyloid beta peptide: what does it take for it to be constructive or disruptive.

Although a major constituent of the senile plaques in Alzheimer's disease (AD), the mechanism of action of the amyloid-β (Aβ) peptide prior to its entrance into a pathogenic spin of harmful consequences still remains a puzzle. Spontaneous or transition metal ion-promoted peptide aggregation has been suggested as an initial event whereby Aβ, a peptide cleavage product normally secreted and processed by cells, turns into a toxic oligomeric and/or fibrillary form. The aggregation process is still unclear, however some evidence exists that an essential part of Aβ toxicity is due to its capacity to generate free radicals. On the other hand, some forms of Aβ have been suggested to protect and rescue neurons from excitotoxic injuries. The dualistic properties of the Aβ peptide as a pro-oxidant foe harbored in amyloid deposits of AD plaques, and as a friendly and constructive antioxidant of purported physiological functions has been suggested but its mechanisms are far from being understood.

In previous studies we have characterized several oxidative stress parameters during the course of $A\beta_{1-40}/Fe^{2+}$-induced apoptotic death in neuronal cells. A marked decrease in protein kinase C (PKC) isoforms, reduced Akt serine/threonine kinase activity, Bcl 2-associated death promoter (BAD) phosphorylation and enhanced p38 mitogen-activated protein kinase (MAPK) and caspase-9 and -3 activation, following addition of Aβ and Fe^{2+} were noticed. These activities, reminiscent of a proapoptotic cellular course were blocked by deferroxamine. In contrast, addition of $A\beta_{1-40}$ alone increased PKC isoform levels, enhanced Akt activity and Ser-136 BAD phosphorylation in accord with an antiapoptotic cellular course. GF, a PKC inhibitor, or wortmannin, a blocker of the Akt pathway, enhanced $A\beta_{1-40}/Fe^{2+}$-induced toxicity while SB, a p38 MAPK inhibitor, prevented cell damage and apoptosis. A reciprocal interaction between pro-apoptotic and non-apoptotic pathways following $A\beta_{1-40}$ or $A\beta/Fe^{2+}$ addition to neuronal cell cultures, supporting the hypothesis that metal ion chelation may be beneficial for AD therapy, was indicated.

The beneficial effects associated with the activation of anti-apoptotic pathways and the notion that the amyloid protein and its degradation peptide products may play a role during brain development, prompted us to extend these studies from *in vitro* to *in vivo* conditions. $A\beta_{1-40}$ was injected via the intraperitoneal route into fetuses and the biochemical consequences on several cellular signaling cascades after a severe episode of *in utero* ischemia were studied in brain tissue. $A\beta_{1-40}$ alone enhanced after one day the levels of glutathione (GSH), glutathione reductase (GR),

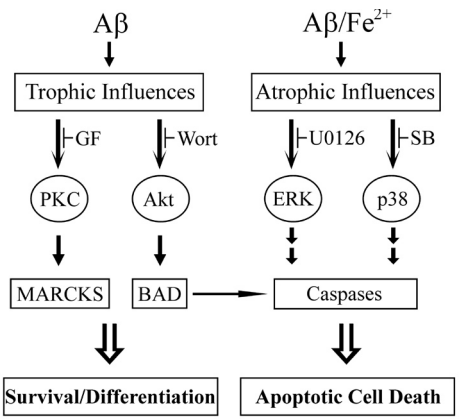

Fig. 1 Activation of apoptotic signalling cascades in cerebral cortex cultures by a mixture of Aβ/Fe and lack thereof by Aβ alone.
Abbreviations used: PKC and its inhibitor GF; Akt; and its inhibitor wortmannin; ERK, and its upstream inhibitor U0126; p38, MAPK and its inhibitor SB; MARCKS, substrate; BAD.

glutathione peroxidase (GPx), as well as stimulated pro-survival signaling activities such as Akt (ERK) and PKC enzymes. Moreover, it reversed the consequences of a transient hypovolemic/ hypotensive stress by restoring GSH levels via its recycling enzymes, by lowering production of lipid peroxides, by activating the aforementioned pro-survival signaling cascades. Reversal of pro-apoptotic consequences was evident by a decrease in p38 kinase phosphorylation, caspases activity and reduced cell death. These data strongly suggest that Aβ may play a critical role in the acquisition of tolerance to free radicals by accelerating the development as well as the effectiveness of the endogenous antioxidants system.

Gene profiling following (n-3) fatty acid dietary deficiency.

The polyunsaturated fatty acid (FA) n-3 docosahexaenoic acid (n-3, DHA) is a most ubiquitous compound in brain and is associated almost exclusively with ethanolamine- and serine- phospholipids that reside in the inner part of the plasma membrane. Experimental studies with animals have demonstrated that depriving the essential n-3 FA precursor, α-linolenic acid (α-LNA) from the diet, reduces markedly the DHA content of cerebral membrane lipids, a process that is accompanied by impairment in behavior, learning ability, sensory motor activity, motivational processes and vision. Furthermore α-LNA deficiency induced abnormal functioning of the mesolimbic and mesocortical dopaminergic pathways. To investigate the molecular mechanism responsible for altering these brain functions we have looked for possible genes that may be associated with these marked changes.

Cross subtracted libraries prepared from total RNA extracts of hippocampus and cortex regions from two-weeks old rats subjected to an intrauterine and early postnatal n-3 FA dietary deficiency were used for identification and profiling of specific genes that may be regulated via dietary supplement of n-3 FAs. A commercial array was used and hybridization of the labeled cDNAs revealed 47 known up-regulated genes. Interestingly, hybridization of a similar RNA preparation without subtraction, revealed only 7 up-regulated genes. Among the over-expressed genes, a group of neurotransmitter receptors related to the dopaminergic system were most pronounced. In addition other neurotransmitter receptors including those for glutamate, serotonin, acetylcholine and GABA were also revealed basically because of the high resolution properties of the subtractive technique used prior to the hybridization. The differential expression of the genes encoding for neurotransmitter receptors was further confirmed by real time RT-PCR. These results suggests that transcription amplified targeting has dramatically improved analysis of differentially expressed genes in nervous tissues particularly for low-abundance mRNA species. Thus, the n-3 FA deprivation during crucial developmental periods may cause an imbalance in the crosstalk and reciprocal interactions among nerve cells.

To extend these studies we have used an in-house microarray containing some 2500 gene sequences generated from a subtractive library of the CA1 rat hippocampus from animals subjected to ischemic stress. Approximately 115 clones were isolated, sequenced, analyzed by the database and clustered using GeneSpring software. In addition to neurotransmitter receptors, genes encoding for G-proteins dependent signaling, various enzymes and cell adhesion molecules were over-expressed. In summary, subtractive libraries appear to have a great advantage by enhancing expression of non-abundant genes. The latter may be part of a repertoire that is highly responsive to seemingly different stress conditions.

Selected Publications

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