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Review Article

Pathophysiology of Brain Aging: A Brief Account on Molecular Changes

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Introduction

Being the second most populous country in the world, India houses a large geriatric population. Increasing geriatric population with increasing age related ailments has necessitated research in the field of Aging. Thus, the study of Biological mechanism of aging is not merely a topic of scientific curiosity, but also a crucial area of research in the current scenario. "Aging" is one of the most fascinating topics that have interested philosophers and scientists for centuries. Over the years, the researchers have postulated several theories to explain the aging phenomena. Denham Harman postulated that aging is a deleterious, progressive, intrinsic, and universal process, which is a progressive accumulation of alteration as a function of time associated with or responsible for the ever-increasing susceptibility to age-related disease and death.[1] Aging is associated with (a) progressive loss of physiologic functions; (b) atrophy to most of the organs; (c) increased susceptibility to infections, trauma and neurodegeneration (d) susceptibility to malignancy, and (e) decreased gaseous exchange during respiration.[2]

More than 300 theories have been proposed to explain the aging phenomenon but no single theory can explain all the mechanisms of aging.[3,4,5] A credent theory of aging should be able to explain the loss of homeostasis in aged individuals; explain the variation in life-span of among cohort genetic strains and species; pinpoint a crucial factor(s) responsible for life-span extension (either by genetic mutation or experimental regimens such as caloric restriction) and demonstrate that any variation of senescent factors could manipulate the rate of aging.[6] Most acceptable explanations for the mechanistic basis of aging was proposed by Harman in 1956 and is called the "free radical theory of aging".[7]

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Free Radical theory of Aging

Harman suggested that aging, as well as the associated degenerative diseases, could be attributed to deleterious effects of free radicals on various cell components. The antioxidant systems are unable to counterbalance the free radicals continuously generated during the life of the cell. This results in oxidative damage in the cell and thus in tissues. Old animals show higher index of oxidation of biological molecules DNA, Proteins and lipids than the young ones owing to increased free radical production.[8.9.10] The discovery of superoxide dismutase enzyme whose function is to remove O2- provided strong evidence that free radicals are involved in the process of aging. Free radical theory of aging lacked the precision of the subcellular location of the oxidative reactions mediated by ROS.

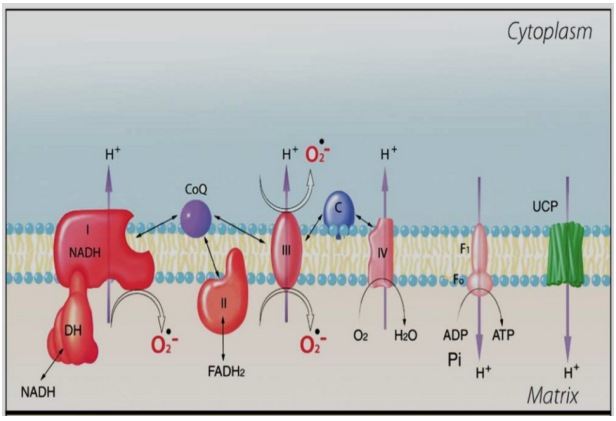
Harman revised the free radical theory of aging to implicate mitochondria as they generated significant amounts of cellular energy through consumption of most of the oxygen in the oxidative phosphorylation.[11] Several studies emerged in support of this theory and since it has been expanded to the mitochondrial free radical theory of aging. Mitochondrial free radical theory of aging proposes that free radicals produced by mitochondria as by-products during normal metabolism result in oxidative damage, and accumulation of oxidative damage is the main driving force for aging. Mitochondrial generation of free radicals is shown in the fig 1.

Age related changes in the Brain

With advancing age, human brain shows anatomical, molecular and functional changes and severity of such alterations results in increased incidence of neurological disorders and TBI's.[13,14] Emerging imaging, genetic and techniques demonstrate the loss of structural integrity, alteration in levels of enzymes, hormones, genetic and epigenetic modulation, dysregulated metabolism, increased

oxidative stress, altered protein processing and synaptic function, and these changes lead to deterioration of physiological and cognitive functions.[15,16,17]

Fig 1. A Schematic Model of ROS Generation in the Mitochondria. The major production sites of superoxide anions at sites I and III are identified along with the major ROS scavenging pathways.[12]



Anatomical Changes

Age-related decline in brain volume is found to be predominant in frontal cortex, temporal cortex, nucleus accumbens putamen and thalamus. MRI analysis has revealed reduction in grey matter volume and increase in white matter volume in frontal, parietal and temporal cortices in both genders.[18,19] Observations have revealed volumetric changes in white matter in different regions of the brain during aging. Some parts of the brain show a decrease in volume by 1% per year which could be due to neuronal shrinkage, reduction of synaptic spines, lower number of synapses and reduced length of myelinated axons.[20,21] Aging is associated with changes in the dendritic morphology, alterations in neurotransmitter receptors and electrophysiological properties. Reduction in the complexity of dendrite arborization and dendritic length occur during aging. Spine numbers are also decreased, and because spines are the major sites for excitatory synapses, changes in their numbers could reflect a change in synaptic densities. The dendritic growth is still possible in old age, similar to reparative processes in synaptic structure.[22] Dendritic branching and length appeared to be greater in aged individuals than in younger adults and people with dementia. With age, there is no significant regression in dendritic length. Dendritic arbors and spines of cortical pyramidal neurons decrease in size and/or number in humans and primates, as a result of age.[23] Similarly, 46 % decrease in spine number and density has been reported in humans older than 50 years as compared with younger individuals. With increasing age, swollen axonal spheroids are found in globus pallidum, pars reticulata of substantia nigra (SNpc), caudal medulla and anterior horns of spinal cord. The age-related changes in the dendritic arbor and dendritic spines of pyramidal neurons in prefrontal, superior temporal and precentral cortices observed in the human brain may probably underlie the first signs of cognitive decline in learning and memory performance noted in normal aging. There is a strong link between dendritic changes and the post-synaptic effects of neurotransmitters. With advancing age, the number of neurons expressing ionotrophic neurotransmitter receptors and the frequency of spontaneous excitatory postsynaptic currents are reduced while the electrical firing pattern in the neurons involved in information processing is perturbed leading to disturbed cognitive performance.[24] The number and size of synapses change during aging and in response to environmental stimuli. During normal aging, the number of synapses may alter depending on the anatomical area.[20,25,26] In areas with decreased number, the size of the synapses increases as a compensatory phenomenon. In contrast to normal aging, a profound fall in synaptic number occurs in case of Alzheimer's disease (AD) in anatomical areas involved in memory and learning and lesser in cases of Parkinson's disease (PD) and Huntington's disease (HD).[27] Molecular changes: Brain aging involves altered levels of neurotransmitters, enzymes, hormones and metabolites.[28] From early adulthood, the levels of dopamine decline by 10% due to loss of dopaminergic neurons between frontal cortex and striatum, or decreased number of dopamine receptors and their reduced binding affinity.[29] Reduction in dopamine is associated with age-related decline in cognitive and motor performance. Loss of synaptic plasticity in the old brain is attributed to reduced serotonin and glutamate levels.[30,31] Activities of enzymes which regulate monoamine neurotransmitters increase with age and may liberate free radicals from the reactions that exceed inherent antioxidant activity.[32] Aging affects the expression of neurotransmitter receptors. It has been shown that the number of neurons expressing certain ionotropic glutamate receptors and N-methyl-D-aspartate receptor subunits is significantly reduced during aging.[33] Quantitative analysis of the distributions of Glu R2 and NMDA R1 in long and short cortico-cortical connections in young and old macaque and patas monkeys revealed a down-regulation of the expression of both receptors with aging. Glu R2 expression was decreased to a greater extent in the prefrontal cortex compared to other areas, such as the temporal cortex, whereas significant reductions in NMDA R1 occurred mainly in the long cortico-cortical projections from the superior temporal cortex.[33] Endocrine changes in the brain

influence aging brain and its cognitive performance. While hormones like growth hormone, thyroxine, melatonin and sex hormones including testosterone, di-dehydroepiandrosterone, estrogen and progesterone decrease during aging, stress hormone cortisol shows significant increase.[34,35,36] Structural and functional changes related to aging are attributed largely to modulation in the level of estrogen and its receptors.[37]

Age related changes in Calcium homeostasis

A consensus emerging from several studies is that in the aged neurons there is an increased Ca2+ release from the endoplasmic reticulum through both the InsP3 and ryanodine-receptors leading to the proposal that the release of ryanodine might be used as a biomarker of functional aging. [38,39] Aged neurons contain more depolarized mitochondria and this affects both the energy balance and mitochondrial Ca 2+ uptake.[40] Decrease in Ca ++ buffering or delayed uptake results in significant increase in the time taken for the relaxation of Ca2+ signals following stimulation. Rise in intracellular calcium levels is countered by rapid calcium sequestering, by calcium binding proteins in the cytosol like parvalbumin, calretinin, calbindin, calretinin, calmodulin, hippocalcin, etc.[41] An age-related disturbed calcium homeostasis could be due to decline in the function of these proteins and transport systems and has been suggested to contribute to age-related neurodegenerative diseases. [42]

Age-related increase in L-type calcium channels is relatively specific for hippocampus pyramidal cells and decrease in NMDA receptor function in hippocampus and frontal cortex, suggesting certain neuroanatomical sites and specific cell populations involving in Ca2+ dysregulation. Cell-specific susceptibility to Ca2+ dysregulation and oxidative stress could explain region specific neuronal death, which characterize neurodegenerative disease.[43]

Changes in gene expression

Gene expression studies in brain across the lifespan reveal alterations in molecules related to stress, inflammation, immune response, mitochondrial functions, growth factors, neuronal survival, synaptic plasticity and calcium homeostasis.[44] Transcriptional profiling of the aging human frontal cortex brains, ranging from 26 to 106 years of age showed that nearly 4% of the genes expressed in the brain are age regulated.[44] Gene expression changes during aging become apparent in middle age and most pronounced after 70 years of age. Several genes involved in synaptic functions that mediate memory and learning were significantly age down-regulated, the list included synaptic vesicle proteins, glutamate receptor subunits, and members of the major signal transduction systems that mediate long term potentiation (LTP). Especially, the synaptic Ca2+ signaling system appears to be affected with reduced expression of calmodulin 1 and 3, calcineurin B´ α, CAM kinase II´ αand IV, and multiple protein kinase C isoforms.[44] Genes involved in vesicle-mediated protein transport and mitochondrial function were prominently downregulated with aging. Prominently age-upregulated class of genes involved stress response genes including antioxidant defense, DNA repair, and immune function. These findings were confirmed in gene expression studies in different cortical areas of the aging human brain.[45] However, extracortical regions, like cerebellum and caudate, showed different patterns. Cellular and molecular changes in the aging brain adversely affects the functions such as attention, speech, sleep, decision making, working and long term memory. Functional aspects of brain are beyond the scope of this dissertation, hence will not be discussed here.

Oxidative Stress in Aging Brain

Mitochondrial Dysfunction

Mitochondria are considered as pacemakers of biological aging as they continuously produce free radicals. Mitochondria contribute to aging through the accumulation of mitochondrial DNA (mtDNA) mutations and net production of ROS. Free radicals generated by mitochondria, or from other sites within or outside the cell, cause damage to cellular and mitochondrial components such as protein and DNA oxidations.[46] As a result, damaged mitochondria progressively become less efficient, lose their functional integrity and release more free radicals, increasing oxidative damage to the mitochondria, and culminating in an accumulation of dysfunctional mitochondria with age. Accumulation of damaged molecules contribute significantly to the aging process.[47] Studey done by Jose V et al observed a decreased respiratory activity of mitochondria with age in liver, muscle, and brain. Transcription of some mitochondrial genes and also Mitochondrial membrane potential was decreased with age in rats and Drosophila.[2,48] Age-related mtDNA changes are observed in the human brain, multiple mutations were detected and their frequency increased with age. Levels of a 5-kb deletion that particularly affects mtDNA COX genes found to increase with age.[49] Another study documented Low abundance heteroplasmic mutations in COX1, a COX subunit gene located on the mtDNA.[50] Studies have shown decreased membrane potential in corti-

cal and striatal mitochondria and in whole brain mitochondria in aged rats.[51,52] which could be due to decreased electron transfer in mitochondra. Slight dip in H+ driven ATP synthesis in aging rat brain was a result of decreased activity of F1-ATPase observed in brain mitochondria isolated from aged rats.[53] Age-related mitochondrial changes include decline in complex I and complex IV activity, with preserved complex II activity. This suggests that mtDNA changes may mediate the observed complex I and IV functional changes since, Complex I and IV are partly mtDNA-encoded, while complex II is encoded by nuclear DNA.[54]

In a study involving rodents, authors have observed 40–65% decreased activities in mitochondrial enzymes such as complex I, complex IV, mtNOS in senescent brains.[55] Isolated mitochondria from the brain of aged rats and mice show increased oxidation products of phospholipids, proteins, and DNA, decreased membrane potential, and increased size and fragility.[54,56] Mitochondria of hippocampus and cerebral cortex are shown to have increased oxidative stress than in whole brain during rat aging.[57] In study done by Petrosillo et al associated cardiolipin (phospholipid required for complex I function) content to complex I activity in rat brain mitochondria: the activity of complex I was reduced by 30% from 24 months aged rats relative to young ones and cardiolipin content was decreased by 31% as function of aging, with significant increase in peroxidized cardiolipin.[58] Further, mitochondria isolated from aged mammalian brain showed decrease in complex IV activity and decreased cytochrome oxidase was observed in human substantia nigra and rat hippocampus.[55,59,60] We alos observed significant loss of complex 1 activity was observed in substantia nigra through aging.[61] Mitochondria from aged animals show structural alterations in mitochondrial membrane, reduced mitochondrial buffering capacity, chronically depolarized state of the membrane (due to increased proton leak) and reduced ATP synthesis.[40] With age there is a decrease in the number of mitochondria, with large bioenergetically inefficient ones replacing the functionally efficient, small-sized forms.[52] however the total volume of mitochondria remains roughly the same in humans.[62] Following neuronal activity, the ATP content falls significantly in aged neurons in contrast to young ones, though the resting levels are comparable. Studies in aging mice show that, the threshold for Ca-induced, cyclosporin-sensitive, Ca release was significantly lower in isolated brain and liver mitochondria. Aging mice exhibited enhanced permeability transition pore activation in lymphocytes, brain, and liver, suggesting increased susceptibility to Ca-dependent cell death (e.g., excitotoxicity, ischemia-reperfusion damage.[63]

Lipid peroxidation

Significant changes in the composition of membrane lipid has been reported in brain aging. Most important changes involved a decreased level in PUFA and an increase in monounsaturated fatty acids in cerebral cortex and cerebellum of aged rats.[64] Moreover, arachidoic acid (AA), along with n-6 and n-3 fatty acids, were also reported to decrease in brains of aged rats accompanied with cognitive deficit.[65] This study suggests that lower levels of AA might be related to the cognitive deficit in rats as AA concentrations were showed to be correlated with the log-term potentiation (LTP). HNE is generally formed as an end product of linoeic acid peroxidation. It forms covalent adduct of histidine, lysine and cysteine residues in proteins to modify their functions.[66] Studies have suggested that the protein bound acrolein could be a potential marker of oxidative stress in aging.[67] Study showed that the amount of HNE-modified protein staining increased logarithmically with aging in human oculomotor neurons.[68] Also, the HNE-modified proteins, along with neurofibrillary tangles, were observed in the senile plaques in aged dogs.[69]

MDA is the most abundant toxic aldehyde formed by peroxidation of AA. MDA can react with amino acids Lysine, tryptophan, histidine and arginine to form adducts. In aged human brain, it was shown that the MDA was increased in inferior temporal cortexes and in cytoplasm of neurons and astrocytes compared to the young controls.[70] In canine model of aging, MDA were increased in the prefrontal cortex of aged brains.[71] In another study it was shown that the basal MDA level was significantly raised by 19% in hippocampus of old rats.[72] Numerous studies mentioned that the generation of MDA in brains increase with age; however, immunohistochemical studies did not detect significant increase of MDA-modified proteins in aged rat brains suggesting that MDA-modified proteins might react with lipid peroxidation products and form cross-links with each other.[73]

Protein oxidation

Many studies have demonstrated an age related increase in the expression of neurofilament protein.[74,75] Increased expression of neurofilament protein makes neurons more prone to the formation of neurofibrillary tangles, a pathological hallmark of AD, and ultimately leads to neurodegeneration and dementia. Several studies indicate that during normal aging there is a widespread increase in protein oxidation. because of the widespread nature of age-related increases in protein oxidation many investigators believe that elevated levels of protein oxida-

tion is a major contributor to cellular aging.[9,76] Free radical modification of proteins may be responsible for the gradual alteration of physiological function and accumulation of altered proteins in aged brain. Here we list out the views of review from Levine and Stadtman[76] (a) in vitro free radical alteration of catalytic activities, thermal stability and sensitivity to photolytic degradation is similar to aging; (b) inducing free radicals to young animals in vivo can change the enzymes to aged-like forms; (c) increasing the life-span of animals by factors or physiological condition can lead to decreases of protein carbonyl levels , and vice-versa; (d) increased levels of oxidized protein in frontal pole and occipital pole concur with the age-related loss of cognitive function; (e) proteins from aged animals are more sensitive to oxidative damage, compared to the proteins from young animals;(f) protein oxidation levels are correlated to increased surface hydrophobicity of protein in aged animals; (g) protein carbonyl levels increase exponentially with age in different animal species and tissues. The review further proposed that free radical induced protein oxidation might be the cause of changes in aging. Our studies in post mortem human hippocampal tissues showed significant elevation in protein carbonyls and nitrated proteins with aging.[77] Increased protein carbonyls were detected in frontal and occipital cortex of aged humans, cortex of Brown-Norway rats and Mongolian gerbils.[79] in forebrain of Wistar rats and in brain homogenates of aged canine models.[71,78,79,80] A study using postmortem samples of substantia nigra, basal ganglia, and prefrontal cortex from neurologically normal subjects, measured protein carbonyls in soluble proteins and found two fold higher levels of protein carbonyls in substantia nigra pars compacta compared to other regions. Authors also observed that carbonyl content of the major proteins in each region was linearly dependent on molecular weight.[81]

It was observed that the increased protein carbonyl level depends not only on its formation, but also the degradation of the oxidized protein.[82] Recent studies demonstrate proteasome dysfunction during the aging of cell cultures in vitro as well as in the tissues of aging animals.[83,84] Increasing evidence suggests that oxidative modification of the proteasome as a possible reason for proteasome dysfunction CNS. HNE, NO, and related oxidative species inhibit proteasome function suggesting that oxidative stress is responsible for the increase in protein carbonyl levels in aging brain, regardless of activity of proteasomes.[85]

Increased protein 3-NT levels were found in the hippocampus and the cerebral cortex of aged rats the CSF of aged human and the subcortical white matter of aged monkeys.[86,87,88] Our study substantiate the previous observations of elevated 3 -NT in hippocampal tissues through aging.[77] Immunohistochemical studies observed most prominent labeling of 3-NT was in prukinje cell layers and molecular layers of cerebellar cortex, as well as in the surroundings of neuropil in cerebellar nuclei of aged rats.[89] Increase in protein 3-NT level is in regionally specific fashion could be attributed to the level of nitric oxide synthase, which produces NO to activate tyrosine nitration, in cells.[87] Another study involving aging model of monkey authors found nitrated α-synuclein within dopaminergic neurons of substantia nigra, and implicated it as a risk factor for synucleinopathies.[90] Protein oxidation leads to increase protein aggregation by increasing protein misfolding, increase protein hydrophobicity, and altering the rate of protein degradation. Also these types of protein modifications promote non-specific protein–protein interactions and then promote the formation of protein aggregates through a variety of covalent and non-covalent linkages.[82] Protein abnormality in age brain was manifested by structure called neurofibrillary tangles (NF) or senile plaques (SP). These protein structures accumulate with age in the neuropil of frontal cortex and hippocampus (Cotman and Peterson, 1989). These plaques (20-50 μm in diameter) are involved in the enlarged axonal and dendritic processes that sprout and dengerate. More over the degenerating neurites was surrounded by extracellular proteinaceous filaments called β-amyloid (Aβ). SP are present in brain of primates, dogs and polar bears, but not rats. NFT are composed of primary microtubule-associated protein tau (tau) and other insoluble proteins (Kosik etal., 1986). NFT are found in normal aged brain, and the number of NFT increased during the ninth and tenth decades of life. NFT were more numerous in medial temporal lobe regions of postmortem brains and may constitute a pathological substrate for memory loss not only in AD but also in normal aging and mild cognitive impairment.[91] The NFT and SP occur within the same stereotyped anatomical regions, both in normal aging and AD and the severity of the lesions increases with age and disease.[25,92,93] Similar to western developing countries, the brains of aged people from India also reveal NFT and SP with a similar frequency and phosphorylated cytoskeletal protein profile.[94] In another study authors used archived paraffin-embedded frontal and entorhinal cortices (10 in each decade from 0 to 79 years of age, 7 in 80-89 decade) with no history of dementia or other neurodegenerative diseases, and examined by silver staining and antibodies recognizing tau protein accumulation. Tau neuronal aggregates were observed in both frontal and entorhinal cortices from the third decade, and extensive staining from 7th decade with maximal Neuropil threads in

the 9th decade. Astrocytic tau accumulation was observed from the 6th decade, predominantly in layer I and subcortical white matter, and increased in number with aging. This study observed an age-dependant pattern of neuronal, extraneuronal and glial tau protein accumulation in non-neurodegenerative sections .[95]

Conclusion

That the brain changes such as the rate of change, the biological age of the brain, and the processes involved are less understood. Cognition and behavioural changes are attributed to the changes that occur at the levels of molecular ageing, intercellular and intracellular ageing and tissue ageing. A number of neurodegenerative diseases are established to be age related. Many studies are cross sectional in nature and have small numbers of participants with wide ranges in chronological age, lack control for risk factors, take no account of education that may improve performance on cognitive tests, and lack assessment with regard to depression that may also affect performance. These are some of the important limitations in studies on the ageing brain. Our understanding of the ageing brain continues to grow but still requires much research that is especially important given the numbers of geriatric populace in society and their potential levels of cognitive impairment and risk of neurodegenerative diseases.

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