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MINIREVIEW

BIOENERGETIC AND OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

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Summary

Aging is a major risk factor for several common neurodegenerative diseases, including Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and Huntington's disease (HD). Recent studies have implicated mitochondrial dysfunction and oxidative stress in the aging process and also in the pathogenesis of neurodegenerative diseases. In brain and other tissues, aging is associated with progressive impairment of mitochondrial function and increased oxidative damage. In PD, several studies have demonstrated decreased complex I activity, increased oxidative damage, and altered activities of antioxidant defense systems. Some cases of familial ALS are associated with mutations in the gene for Cu, Zn superoxide dismutase (Cu, Zn SOD) and decreased Cu, Zn SOD activity, while in sporadic ALS oxidative damage may be increased. Defects in energy metabolism and increased cortical lactate levels have been detected in HD patients. Studies of AD patients have identified decreased complex IV activity, and some patients with AD and PD have mitochondrial DNA mutations. The age-related onset and progressive course of these neurodegenerative diseases may be due to a cycling process between impaired energy metabolism and oxidative stress.

Key Words: mitochondria, oxidative phosphorylation, electron transport chain, oxidative damage, free radicals, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease

Among the most common neurologic diseases are neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). As the elderly population increases, the prevalence of these age-related diseases is likely to increase. The cause of these diseases is not known, and, with the possible exception of PD, there is no treatment that alters the progression of any of these disorders.

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Of the few risk factors that have been identified for these diseases, increased age is the only one that is common to AD, PD, ALS, and HD. For AD, the incidence and prevalence of the disease increase dramatically with age after age 60; one study showed a 47% prevalence for patients over age 85 (1). Two processes that have been implicated in the aging process are mitochondrial dysfunction and free radical-induced oxidative damage (2, 3, 4). In addition to their possible involvement in aging, mitochondrial dysfunction and oxidative damage may play important roles in the slowly progressive cell death that is characteristic of several different neurodegenerative diseases.

Mitochondria and Oxidative Phosphorylation

In different tissues, mitochondria exhibit different sizes, shapes, and densities (4, 5, 6). Each mitochondrion consists of two phospholipid bilayers, the outer membrane and the inner membrane. The space between the inner membrane and outer membrane is the intermembrane space. The area enclosed by the inner membrane is the matrix, which is the site of the tricarboxylic acid cycle. The inner membrane contains the protein complexes that catalyze oxidative phosphorylation. Five different complexes are involved in oxidative phosphorylation: complex I (NADH: ubiquinone oxidoreductase), complex II (succinate: ubiquinone oxidoreductase), complex III (ubiquinol: cytochrome c oxidoreductase), complex IV (cytochrome c oxidase), and complex V (ATP synthase). Complexes I, II, III, and IV constitute the electron transport chain. Mitochondrial DNA (mtDNA) is a circular molecule that encodes for two mRNA molecules, 22 tRNA molecules, seven complex I polypeptide subunits, one complex III subunit, three complex IV subunits, and two complex V subunits. Nuclear DNA (nDNA) encodes for the remainder of the subunits of the oxidative phosphorylation system.

Free Radicals and Oxidative Damage

Free radicals are species that contain one or more unpaired electrons and exist independently (7, 8). Some of the most important free radicals in biological systems are oxygen-centered free radicals, which include inorganic molecules, such as superoxide and hydroxyl radical, and organic molecules, such as alkoxy and peroxy radicals. Through reduction and oxidation reactions, free radicals may damage a variety of macromolecules. Free radical-induced oxidative damage includes DNA strand breaks (9), DNA adduct formation (such as 8-hydroxy-2-deoxyguanosine) (10, 11), lipid peroxidation (12), and the generation of protein carbonyl groups (13, 14). Oxidative damage to macromolecules may alter their function and thereby lead to impaired cellular functioning or cell death.

Interactions Between Oxidative Phosphorylation and Oxidative Damage

There are significant interactions between oxidative damage and mitochondrial energy metabolism, especially the oxidative phosphorylation system. Oxidative phosphorylation generates most of the free radicals in the cell (15). The components that produce the most free radicals are ubiquinone and cytochrome b_{566} of complex III (16). Free radical generation is increased by inhibition of the electron transport chain (15, 17).

In addition to generating free radicals, the oxidative phosphorylation system itself is vulnerable to damage by free radicals. One mechanism by which the oxidative phosphorylation system may be injured by free radicals is through oxidative

damage to the mtDNA. The mtDNA is particularly susceptible to oxidative damage, and all of the polypeptides that are encoded by mtDNA are components of the oxidative phosphorylation system (4, 6). This susceptibility of mtDNA is probably due to its lack of protective histones, limited repair capabilities, and proximity to the electron transport chain (2, 3, 4). The respiratory chain complexes may also be affected directly by reactive oxygen species. In submitochondrial particles, complex I is particularly sensitive to hydroxyl radical and superoxide anion (18). In *in vivo* studies, complex IV is the most vulnerable to peroxidative stress, but complexes I and II are also affected (19, 20). This vulnerability of the electron transport chain complexes may be due to oxidative damage to proteins. Also, since the complexes are membrane-bound and sensitive to the lipid microenvironment (21, 22), oxidative damage to phospholipids of the inner mitochondrial membrane may also be involved.

A cycling process may occur between oxidative damage and oxidative phosphorylation due to the fact that free radicals are injurious to this system that also generates them (Fig. 1). Since oxidative phosphorylation generates free radicals, it is possible that these free radicals damage mtDNA, proteins, and lipids. This damage may then impair oxidative phosphorylation such that greater levels of free

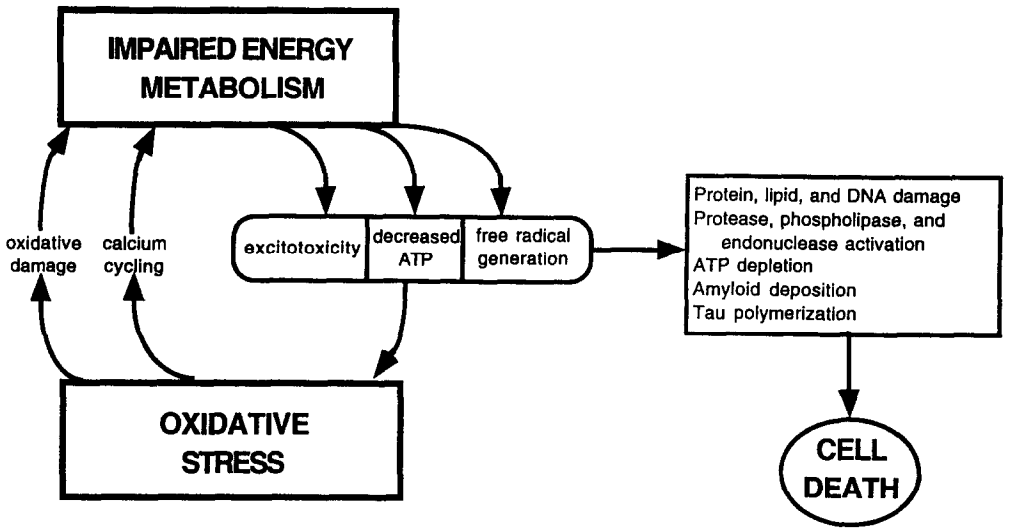

Fig. 1

Possible cycling mechanisms between impaired energy metabolism and oxidative stress. Oxidative stress may produce oxidative damage to macromolecules and "calcium cycling," both of which may impair energy metabolism. Impaired energy metabolism may then result in several processes: excitotoxicity, decreased ATP levels, and increased free radical generation. These three processes may facilitate cycling by further increasing oxidative stress. In addition, these three processes may play an important role in cell death through oxidative damage to macromolecules, excitotoxic mechanisms (protease, phospholipase, and endonuclease activation), ATP depletion, amyloid aggregation, and tau polymerization.

radicals are generated, and these free radicals may result in additional oxidative damage. Another possible cycling mechanism may occur through cytochrome b566; reduced ATP levels result in increased free radical generation by cytochrome b_{566}, such that a slight impairment in oxidative phosphorylation that mildly reduces ATP levels may increase free radical generation, increase oxidative damage to the oxidative phosphorylation system, and then lead to further reductions in ATP levels (23). Also, calcium cycling may play an important role in interactions between energy metabolism and oxidative damage since oxidative stress may impair mitochondrial function due to excessive release and reuptake of mitochondrial calcium (24, 25).

There are several implications of this cycling process between energy metabolism and oxidative damage. First, minor defects that mildly impair oxidative phosphorylation or slightly increase oxidative damage may become amplified over time. For example, an impairment in oxidative phosphorylation may initially produce mildly elevated free radical levels. These free radicals may then impair oxidative phosphorylation further and lead to even greater levels of free radicals. Second, different combinations of environmental and genetic factors that alter mitochondrial function and oxidative damage may produce different rates of amplification and thereby lead to variability in the amounts of oxidative damage observed between individuals. Finally, these cycling processes indicate that the pathway that produces cell death may be similar for defects that initially increase oxidative damage and for defects that initially impair mitochondrial function.

Energy Metabolism, Oxidative Damage, and Excitotoxicity

Impaired energy metabolism and oxidative damage have important interactions with excitotoxicity. These interactions, which have been reviewed recently (26-29), may produce additional cycling mechanisms that are important for cell death (Fig. 1). A full discussion of excitotoxicity is beyond the scope of this review, and, therefore, will be discussed briefly. With regard to the N-methyl-D-aspartate (NMDA) receptor, impaired energy metabolism may enhance excitotoxicity by several mechanisms (26), including a sequence of events that involves decreased ATP levels, reduced sodium-potassium ATPase activity, partial cell-membrane depolarization, and relief of the voltage-dependent Mg^{2+} block of NMDA-associated channels. Also, activation of NMDA receptors involves the generation of nitric oxide (30), which may impair energy metabolism by two mechanisms: (1) inhibition of mitochondrial enzymes, including complex IV (31, 32) and iron-sulfur cluster-containing enzymes, such as complex I, complex II, and aconitase (31, 33, 34, 35); (2) activation of poly (ADP-ribose) synthetase, which may impair energy metabolism and energy-dependent processes by decreasing intracellular levels of NAD and ATP (36, 37).

Free radicals may play an important role in NMDA and non-NMDA receptor-mediated excitotoxicity. NMDA receptor activation has been associated with the generation of superoxide (38). In addition, nitric oxide synthase, which is stimulated by NMDA receptor activation, generates nitric oxide (30) as well as superoxide (39) and hydrogen peroxide (40). These reactive oxygen species may damage and impair the function of a wide range of macromolecules. Also, nitric oxide may react with superoxide to generate peroxynitrite, a powerful oxidant (41). Finally, the NMDA receptor contains a redox modulatory site at which oxidized nitric oxide congeners may react with thiol groups to downregulate channel activity (42). Kainate-induced

neurotoxicity is decreased by antioxidants (43, 44) and is associated with generation of free radicals (45) and increased lipid peroxidation (44, 45), . Also, the toxicity of quisqualate is decreased by idebenone, an antioxidant (46). Free radicals have been associated with increased release (47, 48) and decreased uptake (49) of excitatory amino acids. We recently demonstrated that free radical spin traps can attenuate NMDA-, kainate-, and AMPA-induced lesions *in vivo* (50). Furthermore, we showed that the spin traps decreased hydroxyl radical generation that was produced by these compounds.

Bioenergetic and Oxidative Stress in Neurodegenerative Diseases

Defects in oxidative phosphorylation and oxidative damage may play an important role in a variety of common neurodegenerative diseases, including PD, AD, ALS, and HD. The progressive course and age-related increase in incidence of these disorders may be due to interactions between oxidative damage and defects in oxidative phosphorylation. These processes may be the primary pathologic process or they may be involved secondarily, either as risk factors that facilitate some other primary pathologic process or as processes that become involved "downstream" to a different primary pathologic event.

Aging

Studies in brain and other tissues have suggested that there may be an age-associated impairment of oxidative phosphorylation. Respiratory activity and complex IV activity are reduced in aged rat brain (51, 52), while activities of complexes I and IV are reduced in aged rat muscle (53). Human studies have demonstrated increased numbers of complex IV-deficient myocytes (54, 55), reduced respiratory activity in intact liver mitochondria (56), and decreased activities of several complexes of the electron transport chain in skeletal muscle (57, 58). In a study of the oxidative phosphorylation system in aged rhesus monkeys, we demonstrated an age-related decline in the activities of complexes I and IV in frontoparietal cortex (59). There were no significant age-associated changes in the activities of complexes II-III and V. A study of ATP production in intact mitochondria isolated from aged squirrel monkeys also indicated that there was an age-associated decline in complex I activity that was most prominent in the caudate (60).

In terms of oxidative damage, there is evidence for age-related increases in several different markers of oxidative damage. The most common free radical-induced DNA adduct, 8-hydroxy-2-deoxyguanosine (8OH-dG), increases with aging in nDNA from several different rat tissues (61). The content of 8OH-dG in mtDNA is 10-fold greater than that of nDNA (62) and increases with aging in human diaphragmatic and cardiac muscle (63, 64). In cortical tissue from human postmortem brain, we found a 10-15-fold increase in the 8OH-dG content of mtDNA relative to that of nDNA (65). The amount of 8OH-dG in nDNA and mtDNA increased with aging, and this aging effect was much more striking in the mtDNA than the nDNA.

Aging has also been associated with several different mtDNA deletions. The most common aging-associated deletion is a 4977 nucleotide pair (np) deletion (66-68), but deletions of 7436 np (67-70) and 10422 np (68) have also been reported. The 4977 np deletion may be a marker of oxidative damage since the amount of the deletion correlates with the amount of 8OH-dG (64) and it is increased in cardiac tissue of patients dying with ischemic heart disease (67). Our group has

demonstrated age-related increases in the amounts of the 4977 np deletion in human postmortem brain tissue (70). In this study, the age-associated increase was most marked in the putamen; the cerebral cortex exhibited intermediate levels, and the cerebellum exhibited the lowest levels. Similarly, another group found age-dependent increases in the 4977 np deletion in human brain which were most marked in the caudate, putamen, and substantia nigra (71).

Oxidative damage to proteins results in the modification of amino acid residues (72). Carbonyl derivatives are one of the most extensively studied oxidative modifications (72). In postmortem studies of human frontal and occipital cortex, aging has been associated with a two-three fold increase in protein carbonyl content (14, 72). Free radicals may also damage phospholipids with the formation of lipid peroxides. Studies in brain have demonstrated age-related increases in lipid peroxidation (73, 74). These lipid peroxides may alter the function of lipids or may generate additional free radicals that may damage other molecules, such as membrane-associated proteins.

Recent studies in invertebrates have provided additional evidence for free radical involvement in aging. In *C. elegans*, the age-1 mutation is associated with increased longevity, increased resistance to oxidative stress, and increased activities of catalase and superoxide dismutase (SOD) (75). Transgenic *Drosophila melanogaster* that overexpress catalase and Cu, Zn SOD exhibit increased longevity, decreased protein oxidative damage, and decreased age-associated decline in physical performance (76). In contrast, life-span was not affected or was only minimally increased in transgenic *Drosophila* that overexpress either Cu, Zn SOD or catalase alone (77, 78). Similarly, in mouse epidermal cells transfected with Cu, Zn SOD and catalase, the cells that overproduce only Cu, Zn SOD exhibit increased sensitivity to oxidative stress, while those that overproduce Cu, Zn SOD and catalase are protected from oxidative damage (79). These studies suggest that reactive oxygen species play an important role in the aging process and that significant effects on aging are only observed with concomitant increases in Cu, Zn SOD and catalase activities.

The association of aging with the insidious development of impaired mitochondrial function and increased oxidative damage is consistent with a cycling mechanism in which deficits become amplified over time. These age-related deficits may be components of normal aging that are an inevitable consequence of the toxicity of aerobic metabolism. In humans and animals, aging is associated with the slowly progressive development of cognitive dysfunction (80-82) and physiologic deficits (80). These age-related processes exhibit variability between individuals (80-82). It is possible that some of these processes are due to combinations of genetic and environmental factors that influence mitochondrial function and oxidative damage. In addition, some of the variability in the rate of decline of these processes (80, 81) may be due to variable combinations of these genetic and environmental factors.

Parkinson's Disease

Much of the interest in the association of neurodegeneration with mitochondrial dysfunction and oxidative damage emerged from studies of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (83). MPTP is converted to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B (MAO-B). MPP^+ inhibits complex I activity by binding reversibly to a site at or near the

rotenone-binding site. The close relationship between mitochondrial dysfunction and oxidative damage is apparent with MPP^+ toxicity since MPP^+ induces superoxide formation (84), increases lipid peroxidation (84), and, with prolonged exposure, irreversibly inhibits complex I by a mechanism that may be due to oxidative damage to complex I (85).

Substantial evidence has been obtained of a mitochondrial defect in PD. In accordance with the finding that MPP^+ inhibits complex I, the most consistent oxidative phosphorylation defect in PD patients has been reduced complex I activity. In studies of PD brain tissue, complex I activity is decreased in substantia nigra (86-91) and is not altered in other brain regions (89, 90, 92). An immunohistochemical study showed reduced staining for complex I subunits in PD substantia nigra, but preserved staining for subunits of the other electron transport complexes (93). Similarly, decreased complex I activity has been reported in skeletal muscle (94-96), platelets (97-101), and lymphocytes (102). However, in contrast to these findings, some studies in platelets, muscle, and fibroblasts have demonstrated no reduction in complex I activity (90, 92, 99, 103-106) or reduced activity of other complexes (86, 92, 94-96, 99, 100, 102). Alpha-ketoglutarate dehydrogenase complex (KGDHC), a mitochondrial enzyme that is the rate-limiting enzyme in the tricarboxylic acid cycle and provides succinate for complex II, has also been implicated in PD: KGDHC is inhibited by millimolar concentrations of MPP^+ (107), and immunostaining for KGDHC is decreased in PD substantia nigra (108). Some early studies indicated that the 4977 np deletion of the mtDNA was increased in the striatum and cortex of PD patients (109), but this finding has not been replicated in subsequent studies in substantia nigra (110-112), putamen (111), cortex (111), or skeletal muscle (94, 105, 113).

Evidence for increased oxidative stress in PD includes increased lipid peroxidation in the substantia nigra in PD patients (114, 115). The levels of reduced glutathione are decreased in the substantia nigra but not in other brain regions (116-120). Similarly, in a small number of patients with incidental Lewy body disease, which may be an early stage of PD, the only significant biochemical abnormality was a 35% reduction in reduced glutathione levels (121). In some of these glutathione studies, there may have been autolytic loss of glutathione during the postmortem interval (122). Decreased glutathione levels could produce, or result from, increased oxidative stress. In PD substantia nigra, the activity of γ-glutamyl transpeptidase, a glutathione degradative enzyme, is increased (123), while the activity of glutathione peroxidase may be decreased (124) or unaltered (123, 125). Decreased catalase activity has been reported in PD substantia nigra (126). Conflicting results have been obtained with SOD activity in the substantia nigra. In one study (127), the activity of Mn SOD was increased and the activity of Cu, Zn SOD was not altered; in another study (125), the activity of Mn SOD was not altered and the activity of Cu, Zn SOD was increased. If the activities of Mn SOD or Cu, Zn SOD are actually increased, then this may represent a compensatory response to increased oxidative stress.

It has been hypothesized that increased oxidative stress in the substantia nigra in PD is caused by increased iron levels. Iron could increase oxidative stress by the Fenton reaction, in which hydrogen peroxide is converted to hydroxyl radical (8). Total iron levels were elevated in PD substantia nigra (118, 128, 129). The significance of the findings in PD patients is not clear, however, since the levels of ferritin, which influence the amount of iron that is in a free and reactive form, were decreased in one study (130) and increased in another study (118). The increases

in iron may be secondary since iron is also increased in the substantia nigra in MPTP-treated primates (131).

Amyotrophic Lateral Sclerosis

The strongest evidence for a role for oxidative damage in the pathogenesis of a neurodegenerative disease has recently been obtained in autosomal-dominant familial ALS. Familial ALS accounts for approximately 10% of all ALS cases and exhibits pathologic and clinical features that are similar to those of sporadic ALS (132-134). Familial ALS has been associated with missense mutations in the *SOD1* gene, which encodes for Cu, Zn SOD; 11 different missense mutations were initially identified in exons 2 and 4 in 13 different families (135). Subsequently, several additional familial ALS-associated *SOD1* mutations have been identified, including mutations in exons 1 and 5 (136-140).

Familial ALS patients with several different *SOD1* mutations exhibit decreased SOD activity. In patients with a codon 4 mutation (ala->val), we found a 40-50% reduction in enzyme activity in postmortem brain tissue, erythrocyte lysates, and lymphoblastoid cells (139, 141). In familial ALS patients with several different *SOD1* mutations, erythrocyte lysate SOD activity was decreased by 50-65% (136). Similarly, in patients with a codon 38 (leu->val) mutation, erythrocyte SOD activity was decreased by 66% in an affected patient and by 60% in seven unaffected carriers of the mutation (142). Erythrocyte SOD activity was decreased by approximately 20% in six patients with a codon 46 mutation (his->arg) (137).

These findings in a variety of tissues indicate that the *SOD1* defect is probably generalized, and yet familial ALS preferentially involves motor neurons. The vulnerability of motor neurons may be due to other factors. Excitatory amino acid toxicity has been implicated in the pathogenesis of ALS on the basis of increased CSF glutamate levels (143), decreased high-affinity glutamate transport in postmortem studies (144), and motor neuron degeneration induced *in vitro* by inhibition of glutamate transport (145). In addition, a recent study reported that riluzole, a glutamate release inhibitor, slowed the progression and improved survival in ALS patients with bulbar-onset disease (146). Activation of excitatory amino acid receptors can increase both superoxide and nitric oxide generation, which may then lead to the formation of peroxynitrite (38, 147).

There are several possible explanations for the finding that to date all familial ALS-associated *SOD1* mutations are associated with decreased enzyme activity. Near-maximal Cu, Zn SOD activity may be necessary for the wild-type phenotype, and, therefore, modest reductions in activity may result in disease. However, most dominantly inherited disorders result in a gain of function (148, 149). Thus, it is possible that there is actually a gain of function and that, while the mutations may produce reductions in activity, this reduced activity is not responsible for the disease process. A gain of function is suggested by a recent study in which an ALS-like syndrome occurred in transgenic mice that expressed two wild-type mouse *SOD1* alleles and high copy numbers of mutant human *SOD1* alleles (150). One possible gain of function is that the mutant Cu, Zn SOD reacts more readily with peroxynitrite and thereby generates higher levels of toxic nitronium-like intermediates that lead to increased nitration of tyrosine residues in proteins (151). The function of critical proteins could be altered by these nitrotyrosine residues.

Published studies of oxidative damage and mitochondrial function in familial ALS are limited at this time. In Brodmann area 6 (precentral and supplementary cortex) from patients with a codon 4 mutation (ala->val), we found a 20.5% increase in protein carbonyl derivatives; however, this change was not significant (141). Other brain regions that exhibit more marked pathologic changes, such as Brodmann area 4 or spinal cord, may exhibit more oxidative damage. Complex I activity was elevated significantly by 55.3% in frontal cortex in familial ALS patients (141). These findings indicate that altered mitochondrial energy metabolism may be associated with the pathologic process.

In sporadic ALS patients, SOD activity levels have been determined in a several different tissues. SOD activity was not significantly altered in postmortem brain tissue (141), erythrocyte lysates (141, 142, 152), or muscle biopsies (152). However, cerebrospinal fluid SOD activity, which is primarily Cu, Zn SOD activity, was decreased in two studies (153, 154).

In a study of oxidative damage in sporadic ALS patients, we found that protein carbonyl groups were increased by 84.8% in frontal cortex (Brodmann area 6) (141). Another group (152) reported no significant change in malondialdehyde, a marker of oxidative damage to lipids, in erythrocytes or muscle biopsies from sporadic ALS patients. The density of glutathione binding sites is increased in the dorsal and ventral horns of sporadic ALS patients (155); this could be due to decreased extracellular concentrations of glutathione or altered modulation of the glutathione binding site.

In contrast to our results in familial ALS patients, we did not find any significant alteration in the activities of the electron transport chain complexes in Brodmann area 6 from sporadic ALS patients (141). Ragged red fibers, which are associated with mitochondrial dysfunction in muscle tissue, have been observed in one patient with sporadic ALS (156). Large mitochondria and intramitochondrial inclusions have been observed in hepatocytes from sporadic ALS patients (157-159), and mitochondria with abnormal protrusions have been observed in anterior horn cells of sporadic ALS patients (160). The significance of some of these ultrastructural mitochondrial abnormalities is unclear (161). Support for impaired energy metabolism in sporadic ALS has been obtained in several positron emission tomography (PET) studies that demonstrate widespread reductions in glucose utilization in the cerebral cortex (162-164).

Huntington's Disease

In HD, studies have reported decreased glucose and oxygen utilization in the cortex and basal ganglia of HD patients and some patients at risk for HD (165-171). Recently, with localized proton magnetic resonance spectroscopy, we found increased lactate concentrations in the occipital cortex of HD patients (172). A similar increase in the occipital lactate level has been reported in patients with Kearns-Sayre syndrome, a multisystem neurologic disorder that is associated with deletions of mtDNA (173). In the HD patients, the lactate level correlated with duration of illness (172), and the lactate concentrations decreased when patients were administered coenzyme Q_{10} (174), a compound that may bypass defects in oxidative phosphorylation and act as an antioxidant (174, 175).

Biochemical studies of brain tissue from HD patients have demonstrated multiple defects in the caudate: decreased complex II activity (177); decreased

complex II-III activity and no alteration of complex I or IV activities (178); decreased complex IV activity (179). In platelets, one study reported decreased complex I activity and no change in the activities of complexes II-III and IV (180). Ultrastructural abnormalities in mitochondria have been described in HD cortical tissue (181, 182), and "tweed-ball" mitochondria have been reported in a skin biopsy from a patient with a disease that resembled HD clinically but was not familial (183). Of interest, a point mutation in the ND6 subunit of complex I has recently been associated with basal ganglia degeneration (184).

Studies have not assessed directly whether oxidative damage is altered in HD patients. Lipofuscin accumulation, which may be an indicator of oxidative stress, is increased in frontal cortex (181). In addition, in the basal ganglia, neurons that are vulnerable to degeneration in HD accumulate more lipofuscin than those that are resistant to degeneration (185).

Alzheimer's Disease

In AD patients, PET studies demonstrate reduced glucose metabolism in the temporoparietal region (186-188). These changes occur early in the disease when there is minimal cognitive impairment. Multiple defects in energy metabolism have been identified in AD patients. In brain, adenylate energy charge is unchanged but, since oxygen uptake is increased at submaximal metabolic activity, there may be uncoupling of energy metabolism. In these studies, production of $^{14}CO_2$ from [U-^{14}C]-glucose was also increased (189, 190). Skin fibroblasts from AD patients exhibit decreased glucose utilization (191). Several studies of cortical tissue from postmortem brain samples have also demonstrated decreased activities of pyruvate dehydrogenase complex (192-196) and alpha-ketoglutarate-dehydrogenase complex (KGDHC) (195-197). In skin fibroblasts from familial AD patients, KGDHC activity is decreased by 44% and the E2k component of the enzyme complex contains a polypeptide that is absent or present in minimal amounts in controls (198).

In studies of the oxidative phosphorylation system, AD has been associated with reduced complex IV activity. We found consistent reductions in complex IV activity in four cortical regions and no consistent alteration in the activities of complexes I, II-III, or V (199). Another study reported decreased complex IV activity in frontal and temporal cortex (200). By complex IV histochemistry, the distribution of activity in the molecular layer of the dentate gyrus was altered and activity was decreased in the dentate gyrus and hippocampal subfields (201). A recent study of purified mitochondria isolated from AD brain tissue demonstrated decreased activity of complex IV and no change in the levels of cytochrome aa_3, the heme-containing component of complex IV (202). These findings suggest that the decreased enzyme activity is due to abnormal catalytic activity rather than decreased enzyme levels. In platelets, Parker et al. have demonstrated decreased complex IV activity in mitochondria isolated by two different methods (203, 204). However, with a less pure mitochondrial preparation, there was no significant alteration in complex IV activity (205). If complex IV activity is impaired in AD, then it may result in increased mitochondrial generation of reactive oxygen species. In houseflies, complex IV activity declines with aging and inhibition of complex IV activity is associated with increased hydrogen peroxide production (206). Inhibition of complex IV in submitochondrial particles leads to increased production of superoxide (207).

In an investigation of mtDNA mutations associated with AD and Parkinson's disease (AD-PD), we identified a mutation of a moderately conserved nucleotide (np

4336) in the sequence that encodes for $tRNA^{gln}$; the mutation was present in 5.2% of patients with AD-PD and in 0.7% of controls (208). Mutations of other mtDNA genes, including a complex I subunit gene, the 12S rRNA gene, and the 16S rRNA gene, were identified in AD-PD patients. These preliminary findings are encouraging but need further confirmation.

There is a limited amount of evidence for increased oxidative damage in AD. We recently found a threefold increase in 8OH-dG levels in mtDNA isolated from cortical tissue of AD patients (209). Also, cortex from AD patients exhibits increased lipid peroxidation (210-213). The activity of glutamine synthetase, an enzyme that may be especially vulnerable to oxidative damage, is reduced in frontal cortex from AD patients (14).

Conflicting results have been obtained in studies of antioxidant defense systems in AD. Mn SOD activity was mildly increased in the hippocampus, and Cu, Zn SOD activity was mildly increased in the caudate (214). However, decreased SOD activity has been reported in frontal cortex, hippocampus, and cerebellum (212). Immunoreactivity for Cu, Zn SOD, Mn SOD, and catalase was increased in the region of neurofibrillary tangles and plaques (215). In association cortex and hippocampus from control subjects, high levels of Cu, Zn SOD immunostaining occur in large pyramidal neurons, which are susceptible to degeneration in AD (216). SOD studies in erythrocytes from AD patients have reported no alteration in activity (217, 218) or increased activity (219, 220). In fibroblasts from familial AD patients, SOD activity was significantly increased (221). Erythrocyte glutathione peroxidase activity in sporadic AD has been reported to be normal (217, 218) or increased (222). Fibroblasts from familial AD patients exhibit decreased DNA repair capacity (223), and fibroblasts from familial and sporadic AD patients are more susceptible to oxidative damage (224).

Oxidative stress and mitochondrial dysfunction may interact with other processes that have been implicated in AD pathogenesis. In addition to their possible involvement in excitotoxicity (27), mitochondrial dysfunction and oxidative damage may also facilitate amyloid aggregation and tau polymerization. Amyloidogenic amyloid precursor protein (APP) fragments aggregate in the presence of metal-catalyzed oxidation systems; this effect is inhibited by free radical scavengers (225, 226). In addition, the toxicity of APP fragments in PC12 cells is inhibited by vitamin E and propyl gallate, another antioxidant (227). Amyloid may generate free radicals (228) and inhibit mitochondrial function (229). Oxidation of tau protein appears to facilitate dimerization and polymerization into filaments (230). Also, decreased intracellular ATP concentrations, which could occur with mitochondrial dysfunction, increase the activity of a kinase that may be involved in the hyperphosphorylation of tau (231, 232).

Multisystem Involvement in Neurodegenerative Diseases

If a mechanism involving bioenergetic and oxidative stress is common to the pathogenic process in several different neurodegenerative diseases, then the characteristic clinical and pathologic features of each disease may be due to an interaction with other processes, such as excitotoxicity or amyloid aggregation. Similar underlying mechanisms for these degenerative diseases may result in the coexistence of the features of more than one disease in some patients. In fact, the clinical or pathologic features of more than one neurodegenerative disease have been reported in many patients. Pathologic and clinical evidence of PD exist in AD

patients at a frequency greater that that expected by chance (233). Guamanian ALS-parkinsonism-dementia complex patients exhibit various combinations of motor neuron disease, parkinsonism, and dementia (234, 235). Sporadic ALS and Parkinson's disease occur in combination more frequently than expected by chance (233, 236, 237), and, in sporadic ALS cases with no clinical evidence of parkinsonism, pathologic (238-240) and PET studies (241) indicate that there may be nigrostriatal degeneration. There may be nigrostriatal degeneration in HD as well (242). There are case reports of sporadic ALS in association with AD (243) as well as other forms of dementia (236, 237, 244-246), including "aphasic dementia" (248), frontal lobe dementia (249), dementia with Kluver-Bucy syndrome (250), and Pick's disease (251). Familial ALS has also been associated with dementia (252-254). HD in association with ALS (255) or AD (256) has been described, but these associations are so rare that they may be fortuitous.

Conclusion

Evidence is accumulating that bioenergetic- or free radical-based mechanisms may play an important role in neurodegenerative diseases. While much of the evidence is not definitive at this time, supportive data have been obtained for a wide range of neurodegenerative diseases. For some of these diseases, bioenergetic or oxidative stress may be involved in the primary pathologic event, while for other diseases, these processes may facilitate, or result from, a different primary event. Further studies are required to clarify whether impaired energy metabolism or oxidative damage are involved primarily or secondarily in the pathogenesis of these disorders. For therapeutic considerations, the exact point at which these processes may be involved in cell death may be less important. Whether they are primary or secondary events, mitochondrial dysfunction and oxidative stress may play critical roles in cell death and occur at a time when cells are not irreversibly damaged. If that is the case, then antioxidants and compounds that improve mitochondrial function may slow the degenerative process in several different diseases.

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