

Oxygen, Antioxidants and Brain Dysfunction

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Brain is a logical target of free radical damage, considering the large lipid content of myelin sheaths and the high rate of brain oxidative metabolism. Thus, the hypothesis that free radicals may be involved in the pathogenesis of certain CNS diseases has gained increasing popularity in recent years. In CNS ischemia-reperfusion injury, the role of free radicals appears to be well established, however, involvement of other factors, such as excitatory amino acids and prostaglandins, may also contribute to the production of neuronal necrosis following ischemia. Liberation of free iron appears to play a crucial role in the generation of reactive oxygen species in posttraumatic epilepsy. Although there is no direct evidence to indicate free radical involvement in the pathogenesis of Alzheimer's disease, brain trauma with release of iron, amyloid angiopathy and disturbances in blood-brain barrier function all appear to contribute to the development of ischemic episodes with free radical generation and neuronal degeneration. In Parkinson's disease, the substantia nigra appears to be under oxidative stress as evidenced by the findings of increased lipid peroxidation, reduced GSH levels, high concentration of iron and free radical generation via autocatalytic mechanisms within neuromelanin-containing catecholaminergic neurons. Regardless of the initial insult, a cascade of events involving both reactive oxygen radicals and mitochondrial metabolism is likely to contribute to cell injury.

Key Words: Free radicals, antioxidants, degenerative diseases, CNS

The use of oxygen to produce high energy compounds such as adenosine triphosphate (ATP) is an essential feature of all aerobic life forms. However, oxidative processes also generate highly reactive oxygen free radicals in living tissues. Thus, the interaction between reactive oxygen species and host antioxidant defense systems is a part of normal life and appears to play an important role in normal and abnormal functioning of the central nervous system (CNS).

Molecules or molecular fragments with one or more unpaired electrons are called free radicals (Halliwell and Gutteridge, 1985). A compound becomes a free radical either by losing

an electron or by gaining an additional electron. Free radicals are also formed by homolytic bond fission of covalent bond. When a bond splits symmetrically and leaves each of the two fragments of the molecule with a single electron, they become free radicals (Dormandy, 1989).

Any chemical moiety containing an oxygen atom with an unpaired electron in the outer orbital shell is called an oxygen free radical. Univalent reduction of oxygen or the action of ionizing radiation of oxygen results in the formation of oxygen free radicals. When the first electron transfer occurs on oxygen, the superoxide radical ($O_2^{\cdot-}$) is formed. With the acceptance of the second electron, hydrogen peroxide (H_2O_2) is formed. The third electron transfer results in the formation of hydroxyl radical ($OH^{\cdot}$), the most reactive form among oxygen free radicals.

It has been estimated that about 2% of the oxygen consumed by mitochondria is incompletely utilized and appears as reactive oxygen

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Table 1. Formation of free radicals

$O_2 + e^- \rightarrow O_2^{\bullet -}$	(1)
$AH_2 - e^- \rightarrow AH^{\bullet} \rightarrow A$	(2)

(1) shows the reduction of molecular oxygen to the superoxide anion radical ($O_2^{\bullet -}$).

(2) shows the oxidation of ascorbic acid (AH_2) to dehydroascorbate (A) through a free radical intermediate ($AH^{\bullet}$).

Table 2. Diagram of heterolytic and homolytic covalent bond fission

$A:B \longrightarrow A^- + B^+$	(3)
$A:B \longrightarrow A^{\bullet} + B^{\bullet}$	(4)

(3) shows heterolytic asymmetric cleavage of covalent bond producing negatively charged A ion and positively charged B ion.

(4) shows symmetric homolytic cleavage of bond producing free radicals $A^{\bullet}$ and $B^{\bullet}$, each of these components possessing one unpaired electron.

species (Boveris and Chance, 1973). This proportion may be elevated when efficient functioning of the electron transport system is compromised. Any pathological event involving disruption of membrane structure or mitochondrial function thus has the potential for the induction of free radicals.

Table 3. Potentially cytotoxic reactive oxygen species

$O_2^{\bullet -}$	Superoxide anion radical
$HO_2^{\bullet}$	Hydroperoxyl radical
H_2O_2	Hydrogen peroxide
$OH^{\bullet}$	Hydroxyl radical
$ROO^{\bullet}$	Peroxide radical (R=lipid)
1O_2	Singlet oxygen

The unsaturated bonds of membrane cholesterol and fatty acids can readily react with free radicals and undergo peroxidation. Each lipid peroxide is also a free radical and, once initiated, the process of peroxidation can become autocatalytic as each lipid peroxide attacks a neighboring fatty acid to yield additional lipid peroxide products (Bast and Goris, 1989).

DNA molecules are arranged in a tightly bound helix so that the chance of contact with free radicals is minimized (Slatkin *et al.* 1985). Furthermore, much of the DNA molecule is protected by histones, and if damage does occur, most can be restored by enzyme repair systems. Superoxide radical has been postulated to be involved in triggering breaks in DNA strands (Birnboim, 1988). In double stranded DNA, strand scission may occur as a result of exposure to free radicals resulting in chromosomal deletions or in aberrant gene expression and subsequent cell death (Freeman and Crapo, 1982).

TARGETS OF FREE RADICAL ATTACK

Proteins, membrane lipids and nucleic acids are targets of free radical mediated injury. Free radicals may act by peroxidation of membrane lipids, inactivation of enzymes by oxidation of sulfhydryl groups, depolymerization of polysaccharides, and disruption of nucleic acids.

The aromatic amino acids tryptophan, tyrosine and phenylalanine are the most sensitive because they contain unsaturated bonds (Kleinveld *et al.* 1989). The sulfur-containing amino acids cysteine and cystine are also highly reactive because disulfide bonds and sulfhydryl groups are particularly susceptible to the actions of free radicals.

BRAIN AS A TARGET OF FREE RADICAL ATTACK

Brain contains a high concentration of unsaturated lipids and is a tissue that accounts for about one-fifth of the total oxygen demand of the body. Unsaturated fatty acids in membrane lipids of brain account for more than 20% of the total fatty acids of the brain (Floyd *et al.* 1984). They are also accompanied by relatively low levels of potentially protective enzymes and free radical scavengers. Human brain has about 3% of the total glutathione peroxidase (GPX) of human liver, and there is a complete absence of nonselenium dependent enzyme.

The total superoxide dismutase (SOD) is similar to that observed in heart and liver. Catalase levels are low, less than 1% of those of liver or erythrocytes. There is also less Vitamin E, about one-half that of liver. On the other hand, the brain contains high levels of ascorbic acid. Finally, certain areas of human brain, such as basal ganglia, contain large amounts of total iron. It has been reported that several areas of adult human brain contain over 200 μg iron per gm of wet weight. This is nearly 10 times the level observed in rat and other laboratory animals.

IRON AND OXYGEN FREE RADICALS

Fridovich (1983) demonstrated that $\text{OH}\cdot$ is produced when a reaction takes place between superoxide and hydrogen peroxide (Haber-Weiss reaction). This reaction is most efficiently catalyzed by the presence of ferrous or cupric ion. $\text{OH}\cdot$ is also produced from hydrogen peroxide in the presence of co-factor (Fe^{2+} or Cu^{2+}); this is known as the Fenton reaction.

In general, all iron in human serum is bound to proteins. About two thirds of body iron is found in hemoglobin, with smaller amounts in myoglobin, in various enzymes and in the transport protein transferrin. Iron is stored in ferritin. To promote free radical production, iron must be liberated from proteins or be made mobile by degrading heme proteins to release iron or from ferritin. A small increase in the level of free iron within cells can accelerate rates of oxygen radical production.

Furthermore, studies have shown that the catalysis of hydroxyl radical formation from hydrogen peroxide requires Fe^{2+} ion, Fe^{3+} being ineffective. When iron forms complexes with di- and triphosphate ester nucleotides, Fe^{2+} remains in the catalytically active state. Thus, if iron is available for movement within the cell, it may ligate with di- and triphosphate nucleotides and is easily reduced by ascorbate to the ferrous form, which is very effective in catalyzing hydroxyl formation from hydrogen peroxide (Floyd *et al.* 1984).

The role of iron in ischemia-reperfusion injury in heart and brain was brought into focus by a recent report by Salonen and coworkers (1992). In a large prospective study, they showed that elevated serum ferritin levels constituted a strong risk factor for acute myocardial infarction. Stored iron in the form of ferritin is not essential for life or for preventing anemia but, when liberated, it can promote tissue injury by provoking the iron-mediated Fenton reaction.

HOST ANTIOXIDANT DEFENSE SYSTEMS

Under normal circumstances, a variety of antioxidant defense systems exist to prevent or regulate formation of these highly reactive and toxic moieties. These include SOD, GPX, catalase and other free radical scavengers, such as reduced glutathione (GSH), ascorbic acid, α -tocopherol, β -carotene, and even metal chelators.

ANTIOXIDANT ENZYMES

The SODs are a group of metalloenzymes that catalyze the transformation of the superoxide radical into hydrogen peroxide. A homodimeric CuZn-SODs are located primarily in the cytosol and a homotetrameric Mn-SODs within mitochondria. A homotetrameric glycosylated Cu-Zn-SODs are located within the extracellular spaces. The gene for the human cytosolic SOD is on chromosome 21 and that for the mitochondrial SOD is on chromosome 6.

Catalysis of hydrogen peroxide to H_2O and O_2 is carried out by either GPX or catalase. These

Table 4. Haber-Weiss reaction and fenton reaction

$\text{O}_2^{\cdot -} + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + \text{O}_2$	(5)
$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}\cdot + \text{OH}^-$	(6)
$\text{O}_2^{\cdot -} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}\cdot + \text{OH}^-$	(7)

(5): shows reaction between superoxide radical and ferric ion (Fe^{3+}) to form ferrous ion (Fe^{2+}).

(6): demonstrates Fenton reaction in which hydrogen peroxide in the presence of ferrous ion produces highly reactive hydroxyl radical ($\text{OH}\cdot$).

(7): shows reaction of superoxide radical with hydrogen peroxide to produce highly reactive hydroxyl radical (Haber-Weiss reaction).

enzymes are present in virtually all tissues although catalase levels are much lower than those of SOD or GPX in the CNS. Catalase is localized within peroxisomes, which contain many of the peroxidase generating systems. The activity of selenium-containing GPX is coupled to the production of NADPH via the pentose phosphate shunt. Various other peroxidases exist in biological systems and are especially important in tissues lacking catalase or glutathione-dependent peroxidases. This group includes lactoperoxidase, myeloperoxidase, prostaglandin synthetase and hemoglobin.

FREE RADICAL SCAVENGERS

Glutathione (GSH)

GSH is a tripeptide formed by glutamate, cysteine and glycine (L- γ -glutamyl-L-cysteinyl-glycine). It is the most abundant non-protein thiol in almost all aerobic species (Ross, 1988) and is present in millimolar concentrations intracellularly. Glutathione reductase maintains more than 98% of intracellular GSH in the reduced thiol form, GSH.

The most important portion of the tri-peptide is the cysteinyl moiety. This provides the reactive thiol group for many functions of GSH, including (1) detoxification of exogenous and endogenous compounds, such as reactive electrophiles and peroxidases; (2) reduction of disulfide linkages of proteins and other molecules; (3) the major form of cysteine storage, providing a vehicle for the transfer of cysteine between organs; and (4) leukotriene and prostaglandin metabolism, reduction of ribonucleotides to deoxyribonucleotides, and modulation of microtubule-related processes (Deleve and Kaplowitz, 1990). Oxidative stress is known to deplete GSH reserves (Maellaro *et al.* 1990), and such depletion can cause mitochondrial damage in the brain (Jain *et al.* 1991).

Ascorbic acid (Vitamin C)

Brain contains large amounts (2 mM) of vitamin C. At high concentrations ascorbate will reduce free radicals with the formation of dehydroascorbate. However, at lower concentrations, it may react with transition metals to generate free radicals. As stated above, ascorbate will reduce di- and triphosphate com-

plexed ferric iron to the ferrous form, making it catalytically active. It has not been unequivocally established that a large intake of vitamin C will benefit in health and disease.

Vitamin E (α -Tocopherol)

Vitamin E blocks the chain reaction process that propagates the peroxidation cascade along a membrane. Vitamin E is situated at various sites within membranes near free radical-generating membrane-bound enzymes (Kelly, 1988).

β -Carotene (Precursor of Vitamin A)

β -carotene is accumulated in high concentrations in the membranes of certain tissues such as retina and both quenches excited species and reacts directly with free radicals (Burton and Ingold, 1983; Cotgreave *et al.* 1988). It operates at low oxygen tensions and acts synergistically with vitamin E to inhibit the formation of MDA and block lipid peroxidation (Krinsky, 1988).

Bilirubin

Bilirubin is a toxin in high concentrations but it may serve as an important function as a chain-breaking antioxidant.

Iron and copper-binding proteins

The role of iron and other transitional metals in catalyzing the Fenton reaction has already been stated. There are reports indicating the potentially beneficial effects of the iron chelator deferoxamine in the treatment of brain edema and infarction (Ikeda *et al.* 1989; Patt *et al.* 1990).

Free radicals and neuropathology

Oxidative radicals have been implicated in a broad range of neuropathological conditions for many years. Although there have been some new developments linking free radicals to a variety of CNS diseases, it is extremely difficult to establish whether they actually cause the disorder or represent byproducts of tissue destruction caused by other primary factors.

(1) **Ischemia, edema and infarction:** Many studies have clearly demonstrated the involvement of free radicals in lipid peroxidation and in ischemic neuronal damage. Evidence that free radicals are involved in ischemic damage

can be adduced with the aid of four different methodological approaches. First, we now have methods to determine reactive and partially reduced oxygen species such as superoxide and hydrogen peroxide. The second approach is to determine the amount of known free radical scavenger such as GSH, ascorbic acid and α -tocopherol, and to assume that any reduction in their content results from free radical production. Third, one can assess the accumulation of compounds such as malondialdehyde (MDA), a product of lipid peroxidation and conjugated dienes. Lastly, a common approach is to study whether the damage incurred is ameliorated by the introduction of free radical scavengers.

When 3–6 hours of unilateral carotid artery occlusion is produced in gerbils and circulation is reestablished, the amount of H_2O_2 produced by the affected hemisphere was correlated with edema formation, and the administration of the free radical scavenger dimethylthiourea and of allopurinol (a xanthine oxidase inhibitor and scavenger) ameliorated the edema by reducing free radical formation (Patt *et al.* 1988).

The liberation of iron and heme compounds from hemoglobin following hematoma, hemorrhagic infarction or head or spinal cord injury is acritical factor in the initiation of neuronal death (Braugher *et al.* 1986). Ikeda *et al.* (1989) showed the iron chelator deferoxamine to be protective against cold-induced brain edema. Hall and Braugher (1989) also showed that 21-aminosteroids, specifically developed to quench iron-induced free radical induction, ameliorated spinal cord and brain damage due to trauma and transient ischemia (Braugher and Hall, 1989).

Sakamoto *et al.* (1991) showed, with the electron spin trapping technique, that free radical production and lipid peroxidation was increased during ischemia-reperfusion injury in rat brain. It has also been shown that liposome-entrapped SODs were protective against free radical mediated post-traumatic edema (Chan *et al.* 1987). On the other hand, a study by Agardh *et al.* (1991) showed no indication that variations in the postischemic oxygen supply either altered the production of free radicals or modulated the damage incurred as a result of the ischemia, thus suggesting that free radical production may not be an important factor in the pathogenesis of brain damage following brief periods of ischemia. In their study, regardless

of oxygen tension, ischemic neuronal necrosis was observed in all experimental groups. They recognized, however, that free radical production may be important following longer periods of ischemia.

Output of excitatory amino acids (Pelligrini-Giampietro *et al.* 1990) and prostaglandins were reported to be linked to the production of free radicals in ischemic neuronal damage. Indomethacin treatment is reported to reduce ischemic neuronal damage (Sasaki *et al.* 1988). Indomethacin is an inhibitor of phospholipase A_2 and cyclooxygenase, and thereby reduces free radical formation and inhibit calcium influx.

(2) Seizure disorders: Plaques jaunes characterize post-traumatic scar formation in the cerebrum and may serve as epileptogenic foci. The content of reactive oxygen species in the brain can be elevated by seizure activity (Armstead *et al.* 1989). The addition of iron salts to membrane suspensions (Subbarao and Richardson, 1990) or their injection into rat isocortex (Willmore *et al.* 1983) results in the formation of free radicals and the generation of lipid peroxidation products. Epileptiform electroencephalographic discharges in iron-induced epilepsy have been postulated to involve membrane lipid peroxidation initiated by free radicals. They can be prevented by the addition of antioxidants. It has also been suggested that during ferric chloride-induced epileptic discharge the brain is protected against peroxidative damage by the pentose phosphate pathway, through the generation of NADPH for the reduction of GSH (Singh and Pathak, 1990).

(3) Aging: One of the hallmarks of aging in the brain is an accumulation of lipofuscin in neurons. This aging pigment accumulates as the result of conjugation of aldehydes such as MDA with primary amine groups of other lipids, nucleic acids and proteins to form Schiff's base type compounds. Accumulation of lipofuscin with age is due to either its increased rate of formation or its decreased rate of decomposition or both.

One theory of aging states that free radicals generated during normal cellular metabolism cause accumulation of damage to DNA and other macromolecules. This was modified to state that free radical and free radical reactions are involved in the etiology and development of a number of diseases, especially those that are life-limiting, such as atherosclerosis,

liver cirrhosis and cataract formation (Harmon, 1987; Pryor, 1987).

There is a significant correlation between the ratio of CuZn SOD activity to specific metabolic rate and maximum life span potential in 13 mammalian species, those animals with higher relative SOD activity living longer. Ingestion of antioxidants increase longevity. Addition of mercaptoethylamine to the diets of mice increased life span by 30%. Vitamin E may do the same. There is also a correlation between metabolic rate and life span.

The monoamine oxidase B inhibitor deprenyl, given to rats on a daily basis starting at the end of their second year, is reported to increase their life span significantly (Knoll, 1988). This appears to indicate that deprenyl provides protection against the self-produced neurotoxins associated with dopaminergic neurons in the nigrostriatal pathways. Would augmentation of the antioxidant system help prolong longevity?

It has also been suggested that aging pigments such as lipofuscin may serve as a reservoir for potentially neurotoxic substrates. Do we need to prevent the build-up of this toxin so that neuronal life may be prolonged?

On the other hand, with the use of a fluorescent probe, dichlorofluorescein, LeBel and Bondy (1991) demonstrated a significant age-dependent decrease in the velocity of oxygen radical generation in rats of different ages. However, there was also evidence of protein damage and an increased rate of intracellular proteolysis in aged animals. The age-dependent decrease in cerebral oxygen radical generation coincided with an age-dependent increase in superoxide dismutase. No age-related alterations in lipid order in either the hydrophilic or lipophilic membrane regions were observed using fluorescence polarization analysis. These findings suggested that aging does not proceed as a result of elevated rates of generation of oxygen radicals but modifications in proteins and the activation of protein catabolic pathways may constitute major contributing factors in the normal physiological process of aging (Bondy, 1992). Undoubtedly, oxidative stress in the brain accumulated over a lifespan through normal living, diseases, and environmental or dietary causes may play a significant role to senescence but not as the sole factor.

(4) Alzheimer's disease (AD): There is no direct evidence for involvement of free radicals

in AD pathogenesis. The age-related decline in mental functioning seen in AD suggests that progressive neuronal dysfunction and cell death related to the passage of time underlie the disease process. Association of free radicals and AD pathology perhaps may be suspected when brain injury results in the release of free iron and formation of free radicals. Brain trauma is a risk factor for AD, and the development of AD pathology in a young male after a brain injury has been described. Neurofibrillary tangles observed in dementia pugillistica are identical to those present in AD. Amyloid microangiopathy, blood-brain barrier (BBB) dysfunction may contribute to repeated ischemia and reperfusion of the affected tissue, thus increasing free radical formation and produce focal areas of neuronal degeneration. AD changes occur early in Down's syndrome (DS) cases. The gene encoding for SOD is located on chromosome 21 and is over-expressed in DS patients because of chromosome 21 trisomy. Increased SOD activity may result in increased conversion of superoxide to hydrogen peroxide, which is the substrate for the Fenton reaction. There have been reports of abnormalities in iron-handling and in iron-induced lipid peroxidation in AD autopsy brain tissue (Richardson *et al.* 1990; Subbarao *et al.* 1990; Andorn *et al.* 1990). The beneficial effects of deprenyl in AD have been reported (Tariot *et al.* 1988). The involvement of free radicals in the pathogenesis of AD remains speculative at the present time.

(5) Parkinson's disease (PD): Catecholaminergic neurons containing neuromelanin, an autooxidation byproduct of catecholamines, are more vulnerable in PD than nonmelanized catecholaminergic neurons. It is hypothesized that, in aging dopamine (DA) neurons, the deamination of DA by monoamine oxidase (MAO)-B, which increases with aging, may result in increased formation of hydrogen peroxide and/or other toxic byproducts such as hydroxyl radicals, superoxide, 6-hydroxydopamine or quinones. There have been reports suggesting the beneficial effects of MAO-B inhibitor deprenyl in Parkinson's disease patients (Shoulson, 1989a, b).

The concept that free radicals may be involved in initiating the pathology of PD is supported by the following findings:

(a) Increased lipid peroxidation is evidenced by increased levels of MDA and lipid hydro-

peroxides, while many of the protective mechanisms against oxidative stress are not altered in the substantia nigra of PD patients (Dexter *et al.* 1989a). The activities of GPX and catalase are more or less unchanged, as are the concentrations of vitamin C and vitamin E. The increased activity of mitochondrial superoxide dismutase, the elevated levels of the antioxidant ion zinc and high levels of CuZn-SOD mRNA in the substantia nigra all indicate the presence of oxidative stress in the substantia nigra of PD patients.

(b) Increased iron content in the substantia nigra as compared to other regions of the brain has been reported (Dexter *et al.* 1989b). Is loss of homeostatic control of iron stores involved in Parkinson's disease? Plasma levels of ferritin are increased in aging. Increased brain levels of ferritin have also been reported in Parkinson's disease (Reiderer *et al.* 1988).

(c) A deficiency of mitochondrial complex I activity was also reported (Shapira *et al.* 1990, 1992).

(d) Levels of GSH are decreased in substantia nigra in PD (DiMonte *et al.* 1992). This decrease does not occur in other brain areas or in other neurodegenerative illnesses affecting this brain region. Altered glutathione metabolism may prevent inactivation of hydrogen peroxide and enhance the formation of toxic hydroxyl radicals. Diffuse Lewy body disease brain also demonstrated decreased GSH levels in the substantia nigra (Jenner *et al.* 1992). Although most GSH is localized in the cytosolic fraction, approximately 10% of the total cellular GSH is compartmentalized within mitochondria. Because mitochondria also contain GPX, glutathione reductase and NADPH, a complete system for detoxifying hydroperoxides is present within these organelles. The protein thiols are essential for a number of functions of these organelles, including selective membrane permeability and Ca^{2+} homeostasis.

Thus, excessive production of hydrogen peroxide within mitochondria may lead to depletion of mitochondrial GSH, oxidation of protein thiols, and impairment of mitochondrial function. This relationship may have relevant implications in terms of the degeneration of dopaminergic neurons, because substrates of MAO may be sources of hydrogen peroxides within mitochondria and may cause a decrease in mitochondrial GSH.

Loss of GSH by injection of buthionine sulfoximine (BSO), an inhibitor of glutamylcysteine synthetase, has been shown to cause some kind of mitochondrial degeneration (Jain *et al.* 1991). If loss of GSH may cause mitochondrial damage, it is also conceivable that impairment of mitochondrial function can lead to a decrease in cytosolic GSH. GSH synthesis requires ATP, and thus a deficiency of energy supplies by mitochondria is likely to affect the cellular turnover of GSH.

Regardless of the initial insult, a cascade of events involving both oxygen radicals and mitochondrial metabolism is likely to contribute to cell injury.

Most GSH in the brain is localized in glial cells rather than within neurons thus raising the possibility that glial cells play a more active role in human neurological disorders than previously suspected.

CONCLUSION

Generation of reactive oxygen species is a part of normal life, and their interaction with host antioxidant defense systems appears to exert a significant influence on cellular chemistry in health and disease. Brain is a logical target of free radical attack because of its large concentration of unsaturated lipids and high rate of oxidative metabolism. Oxidative radicals have been implicated in a broad range of neuropathological conditions; however, it is extremely difficult to establish whether free radicals actually cause the disorder or are byproducts of tissue destruction caused by other primary factors. Nevertheless, the involvement of free radicals in a number of CNS diseases appears to be well established. Recently, Rosen *et al.* (1993) demonstrated that a familial variant of amyotrophic lateral sclerosis (ALS) is linked to defects in the SOD1 gene, which encodes the cytosolic SOD. Although pathogenesis of selective neuronal damage of motor neurons brought about by mutations in SOD1 is not clear, it has been postulated that either the reduction in SOD1 activity leading to an accumulation of the toxic free radicals or the increase in SOD1 activity resulting in excessive levels of hydrogen peroxide and generation of highly toxic hydroxyl radical through the reaction of

hydrogen peroxide with a transition metal such as iron. It has also been suggested that physiological activation of excitatory amino acid receptors on motor neurons would generate a low level of superoxide and with limited repertoire of superoxide scavengers such as SOD may then lead to death of motor neurone (McNamara and Fridovich, 1993). Superoxide is released abundantly during the respiratory burst of activated phagocytic leukocytes and plays an important role in inflammation. However, the harmful effects of excessive free radicals in the brain must be controlled. To this end, attempts should be made to obtain precise information concerning free radical generation in specific cells and organelles at those pathologic foci where the principal changes are observed. The ultimate goal is to develop effective pharmacological agents that can control the harmful effects of free radicals without disrupting their essential functions.

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