Oxidative processes and antioxidative defense mechanisms in the aging brain[1]

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ABSTRACT **The debilitating consequences of age-related brain deterioration are widespread and extremely costly in terms of quality of life and longevity. One of the potential major causes of age-related destruction of neuronal tissue is toxic free radicals that are a natural result of aerobic metabolism. The brain is particularly susceptible to free radical attack because it generates more of these toxicants per gram of tissue than does any other organ. The major defense mechanisms the brain uses to combat reducing equivalents is via their enzymatic metabolism. The vitamin antioxidants, vitamin E (α-tocopherol in particular) and vitamin C (ascorbate), also aid in protecting the brain from oxidative stress by directly scavenging toxic radicals. A newly discovered, potentially highly important antioxidant in the brain is the indole melatonin. The pineal hormone melatonin is rapidly taken up by the brain. In vitro melatonin is more effective than glutathione in scavenging the highly toxic hydroxyl radical and also more efficient than vitamin E in neutralizing the peroxyl radical. Furthermore, it stimulates the main antioxidant enzyme of the brain, glutathione peroxidase. In vivo melatonin is a potent antioxidant and it lacks prooxidant actions.—Reiter, R. J. Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J.* 9, 526–533 (1995)**

Key Words: antioxidants • free radicals • oxidative damage • vitamin E • vitamin C • melatonin • neurodegenerative diseases

THERE ARE OBVIOUSLY A VARIETY OF AGE-RELATED neurodegenerative conditions that, in their advanced stages, are debilitating. Some of the most conspicuous and well-known examples of degenerative conditions of the central nervous system whose frequency of occurrence increases during aging include Parkinson's disease, Alzheimer's disease, multiinfarct dementia, and amyotrophic lateral sclerosis. Although extensively studied in terms of their causative factors, there is no definitive consensus on the etiology of any of these conditions. These diseases commonly are associated with movement and/or severe cognitive impairments; in some cases, significant overlay in the morphopathology of these conditions occurs.

That aging in general (1, 2) and aging of the central nervous system in particular (3, 4) may, in part, relate to the damage inflicted by oxygen free radicals and their intermediates has considerable experimental support. Oxidative stress is the usual phrase used to identify the association of toxic free radicals with damage to cells and tissues (5). Ideally, free radicals and free radical generating processes would be neutralized by antioxidative defense mechanisms, of which there are a variety in organisms (6–8). During aging, however, free radical generation may increase as a consequence of normal aging processes, exposure to toxins and ultraviolet light, and stress; alternatively, the defense systems that evolved to combat oxidative stress (e.g., antioxidative enzymes, free radical scavengers, metal chelating agents) may diminish (9–11). The result of these changing processes is an accelerated rate of accumulated damage and associated pathophysiology in advanced age.

It normally would be assumed that, considering its importance to organismal physiology and survival, the central nervous system would be amply endowed with defense mechanisms to counter oxidative stress. Contrary to this expectation, however, the brain is highly vulnerable to attack by oxygen free radicals and it does not have an abundance of processes to neutralize these molecular renegades. Because of its high metabolic activity, the brain uses large amounts of molecular oxygen (dioxygen, O_2).[3] In a conscious young individual, O_2 is used at a rate of 3.5 ml/100 g neural tissue/min (12). To put this into perspective, the brain, which accounts for only 2% of the body weight, uses 20% of the total inspired O_2 in a resting individual. In neural tissue, O_2 primarily participates in the oxidation of carbohydrates and generates an estimated steady turnover of roughly 4×10^{21} molecules of ATP/min in the total brain (12). Despite the brain's reliance on O_2, elevated pO_2 may induce convulsions that are a consequence either of the reduction in the activity of the enzyme glutamate decarboxylase or the increased generation of oxygen free radicals (13). Because a small portion ($<5\%$) of the O_2 used by cells obviously is not involved in the production of ATP, it is used in an alternate pathway, i.e., it is reduced to reactive oxygen species that may induce tissue damage (3, 5, 6, 13). Because the utilization of O_2 is high in the brain, it follows that generation of damaging oxygen species is also elevated. Inasmuch as the brain does not

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[3]Abbreviations: O_2, dioxygen; PUFA, polyunsaturated fatty acids; CSF, cerebrospinal fluid; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; ·OH, hydroxyl radical; $O_{2^{\cdot}}$, superoxide anion radical; MAO, monoamine oxidase; DA, dopamine; CAT, catalase; GSH-Px, glutathione peroxidase; ROO·, peroxyl radical; MDA, malondialdehyde; NO·, nitric oxide; NOS, nitric oxide synthase; $ONOO^-$, peroxynitrite; GSSG, oxidized glutathione; GSH-Rd, glutathione reductase.

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have an excess of protective mechanisms against the reactive derivatives of O_2, the damage left in the wake of these aggressive radicals is probably higher than elsewhere in the body.

The high utilization of O_2 is not the only reason the brain suffers more than its share of oxidative destruction. The brain is highly enriched with polyunsaturated fatty acids (PUFA). The unsaturated bonds in these molecules render them susceptible to oxidative damage by free radicals (14). Cerebrospinal fluid (CSF) contains little transferrin or cearuloplasmin; these compounds serve as antioxidants because of their ability to bind iron and copper, respectively; these transition metals can be used in the generation of the most toxic oxygen-based radical, the hydroxyl radical (·OH) (15). Furthermore, CSF contains low molecular weight iron and copper complexes that can catalyze free radical generation. Beyond this, there are other reasons why the brain exhibits a high susceptibility to oxidative damage. Neural tissue itself contains high concentrations of nonheme iron, which as noted before is involved in the generation of the highly toxic ·OH (16). The elevated levels of iron coupled with high concentrations of ascorbic acid in the central nervous system is another factor that puts the brain in jeopardy of damage by reactive oxygen species. Although generally considered an antioxidant, ascorbic acid in the presence of free iron can be a prooxidant (17). The free iron in this case is often derived from hemoglobin. Hemoglobin, normally transported in erythrocytes, sequesters iron and renders it unavailable for free radical reactions. However, hemoglobin outside of the erythrocyte, e.g., after the breakdown of the cells, potentially is highly dangerous because it can be degraded and liberate iron (18). Thus, in the event of hemorrhage into the brain, e.g., during stroke, the neural damage due to free radical attack can be extensive (19).

Finally, excitatory amino acid neurotransmitters, which are abundant at some locations in the brain, generate massive numbers of free radicals after their release (20) (**Fig. 1**). Glutamate receptor-mediated excitotoxicity is well known to be a causative factor in a variety of neurological disease states. Glutamate, which is the chief excitatory neurotransmitter in the brain, is distributed unequally in the central nervous system. Thus, the damage caused by this neurotransmitter, which is secondary to increased oxygen radical production, is concentrated in those neural structures that contain the highest density of glutamate containing neuronal endings (4, 21). With increased longevity, the damage related to glutamate release accumulates, leading to the gradual morphological and physiological destruction of those neurons that are normally bombarded by this excitatory amino acid neurotransmitter (4).

Unquestionably, the wear and tear on the brain inflicted by toxic free radicals over a lifetime is substantial, with the gradual deterioration of neurobiological tissue being inevitable. Surely the drop in the functional efficiency of the brain, e.g., slowed reactions, diminish memory, tremor, and gross deterioration of some neuronal functions, is in part a consequence of the necessity of most organisms to rely on oxygen for their metabolism. The oxygen free radical damage the brain experiences is part of the price organisms pay for using this toxic environmental molecule.

OXIDATIVE PROCESSES

Twenty-one percent of the gaseous atmosphere of the Earth is O_2. Because of its ability to readily accept electrons (e^-), O_2 is a powerful oxidizing agent. Within atoms and molecules, e^- occupy regions known as orbitals with each orbital capable of containing two e^-s (a diradical), one of which spins in a clockwise direction and the second in an anticlockwise direction. When an orbital contains only a single e^-, the e^- is said to be unpaired, and an atomic or molecular species with an unpaired e^- is referred to as a free radical. O_2 qualifies as a radical because it possesses two unpaired e^-, each in a different orbital and both spinning in the same direction (**Fig. 2**). Within cells, the majority (>90%) of the O_2 in aerobic organisms is utilized by mitochondrial cytochrome oxidase, which adds four e^-s, one at a time, to O_2 resulting in the formation of two molecules of water. O_2 reacts sluggishly with nonradicals, which constitute most biomolecules, because of the phenomenon known as spin restriction. Because O_2 contains two unpaired e^-s with the same spin direction, it can only accept a pair of e^- from a molecule that has two unpaired e^-s with the opposite spin of those in the O_2 molecule. To achieve this spin conversion, groundstate O_2 must react in a two-step, energy-dependent process.

The acceptance of a single e^- by O_2 results in the formation of the superoxide anion radical (O_2^- or dioxide) (22) (Fig. 2). In biological systems, O_2^- production is usually mediated by the one e^- reduction of O_2 by enzymes. Its major source of production is via the activity of mitochondria and microsomal electron transport chains (23). Even though cytochrome oxidase usually tightly binds partially reduced oxygen intermediates to its active site, the chain leaks some e^- directly onto O_2 resulting in the formation of O_2^-. This "accidental" O_2^- generation increases as the O_2 concentration rises because of the associated rise in e^- leakage (24). Also, epinephrine and norepinephrine undergo metal ion-dependent oxidation with the resultant generation

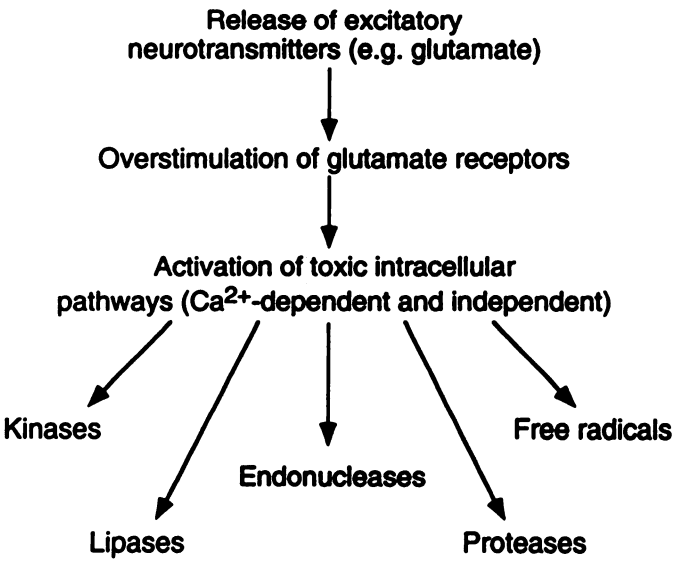

Figure 1. The release of excitatory amino acid neurotransmitters, such as glutamate, induces a cascade of responses in the postsynaptic neuron including the production of toxic free radicals. With time, the repeated stimulation of the neurons with the resultant generation of free radicals takes its toll with the gradual accumulation of damaged molecules and organelles in the postsynaptic neuron. In the worst case, the damaged neurons may die, and because they are not generally replaced, functional deficits arise.

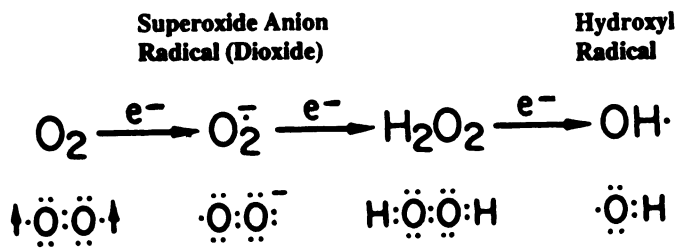

Figure 2. The three-electron (e^-) reduction of molecular ground state oxygen to the hydroxyl radical. The arrows (both pointing in the same direction) associated with the oxygen molecule indicate the two unpaired e^-, located in different orbitals, which have the same spin direction.

of O_2^- (25). Finally, several oxygen-utilizing enzymes including tryptophan hydroxylase, indoleamine dioxygenase, and xanthine oxygenase directly generate O_2^-. Certainly evidence supporting the superoxide theory of O_2 toxicity is substantial (13, 14). However, despite the obvious highly damaging effects of oxygen radicals, they are not always unwanted culprits. For example, activated phagocytes produce O_2^-, which is important in the mechanisms by which these cells engulf and kill bacteria (26); also, low levels of oxygen radicals in some cells act as second messengers in the activation of a cytoplasmic form of the transcription factor NF-κB (27). Indeed, the less-than-perfect antioxidative defense mechanisms may have evolved specifically to permit these necessary functions of oxygen-derived radicals.

Although O_2^- itself is not highly toxic to macromolecules (where oxygen toxicity is usually assessed), it is readily converted to more toxic species. Its low toxicity is reflected in its relatively longer half-life compared with that of the highly toxic ·OH (**Table 1**). The half-life of oxygen radicals is inversely related to their reactivity with each other or with other molecules.

O_2^- is readily dismutated in the presence of the enzyme superoxide dismutase (SOD) with the resultant production of hydrogen peroxide (H_2O_2) (28). Because SOD metabolizes a free radical, O_2^-, to a nonradical species, H_2O_2, it is usually classified as an important component of the antioxidative defense system. However, excessive expression of the SOD gene, such as in individuals with Down syndrome, leads to oxidative damage, premature aging, and neurodegeneration (29) because the H_2O_2 produced as a consequence of the dismutation process is converted to highly toxic radicals (Fig. 2).

Besides SOD, several other enzymes generate H_2O_2; these include L-amino acid oxidase, glycolate oxidase, and monoamine oxidase (MAO). The accelerated oxidation of dopamine (DA) by MAO in the nerve terminals of the dopaminergic axons from the substantial nigra has been suggested to increase H_2O_2 generation in these neurons, thereby elevating oxidative stress (**Fig. 3**) (25). This may lead to a production of highly toxic oxygen species beyond the capability of the antioxidative defense mechanisms of the DA neurons to cope with them, thus leading to the eventual destruction of these neurons. Even the side effects of prolonged L-DOPA therapy in individuals with Parkinsonism have been theoretically linked to elevated H_2O_2 formation (30).

TABLE 1. *Estimated intracellular half-life of some free radicals*

Radical species	Symbol	Half-life at 37°C, s
Molecular oxygen (dioxygen)	O_2	$>10^2$
Singlet oxygen	$^1O_2$	1×10^{-6}
Superoxide anion (dioxide)	O_2^-	1×10^{-6}
Hydoxyl	HO·	1×10^{-9}
Peroxyl	ROO·	1×10^{-2}
Alkoxyl	RO·	1×10^{-6}

Although H_2O_2 is an oxidizing agent, its reactivity is low. Only when present in high concentrations is it normally considered toxic. Unlike O_2^-, however, H_2O_2 crosses cell membranes easily, thereby making itself available for radical reactions at sites distant from where it was formed. Because it has no unpaired e^-, H_2O_2 is not itself a free radical and is usually identified as a reactive oxygen intermediate. In most cells there are two enzymes that detoxify H_2O_2, i.e., catalase (CAT) and glutathione peroxidase (GSH-Px) (Fig. 3). In the brain, GSH-Px is more important than CAT in performing this detoxification task (31).

Although the excessive production of H_2O_2 in neurons of Parkinson's subjects may be considered toxic, it is not the toxicity of H_2O_2 per se that creates the major problem. Rather, H_2O_2 in the presence of Fe^{2+} (or Cu^{1+}) is readily converted, via the Fenton reaction, to the highly oxidizing, molecule-destroying ·OH (32) (Fig. 3).

If the function of radicals is to destroy molecules and tissues, then ·OH would be identified as the radical's radical.

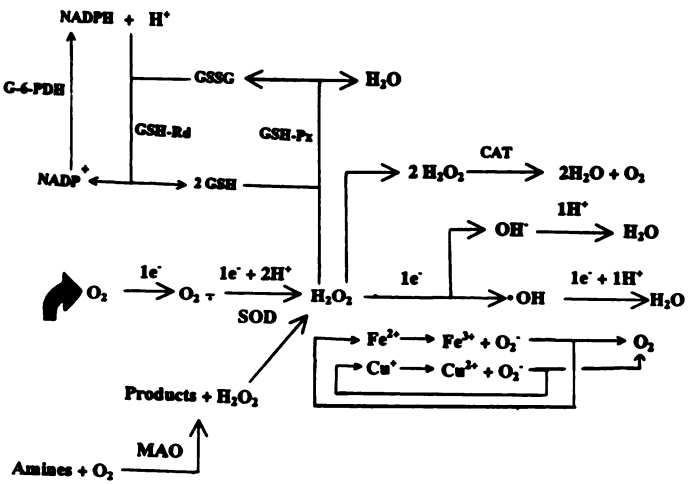

Figure 3. Molecular oxygen (large arrow) can be reduced to the superoxide oxide anion radical (O_2^-), which has a number of fates. O_2^- eventually is converted to toxic oxygen-radicals, so this scheme is collectively known as the superoxide anion theory of oxygen toxicity. Superoxide dismutase (SOD) catalytically reduces the O_2^- to hydrogen peroxide (H_2O_2), which can be reduced in the presence of transition metals (Fenton reaction), usually iron (Fe^{2+}) and sometimes copper (Cu^+), to the highly toxic hydroxyl radical (·OH). Once oxidized, Fe^{3+} and Cu^{2+} can be converted to Fe^{2+} and Cu^+, respectively, in several reactions. H_2O_2 also is enzymatically degraded by either glutathione peroxidase (GSH-Px) or catalase (CAT) to nontoxic products. GSH-Px, in the process of H_2O_2 metabolism, converts reduced glutathione (GSH) to the oxidized, disulfide form (GSSG); the latter is recycled to GSH by glutathione reductase (GSH-Rd). Amine metabolism in the brain by the enzyme monoamine oxidase (MAO) contributes to oxygen toxicity by generating H_2O_2.

It reacts at diffusion rates (Table 1) with virtually any molecule found in its path including macromolecules such as DNA, membrane lipids, proteins, and carbohydrates (33). In terms of DNA the ·OH can induce strand breaks as well as chemical changes in the deoxyribose and in the purine and pyrimidine bases (34).

Membrane lipids also succumb easily to the devastating actions of the ·OH (**Fig. 4**). Lipid peroxidation is initiated by the abstraction of a hydrogen atom from a PUFA side chain in the membrane lipid. As already mentioned, the high vulnerability of neural tissue to oxidative damage is due in part to its high lipid content (14).

The abstraction of a hydrogen atom from a PUFA leaves in its wake a carbon-centered lipid radical. After the molecular rearrangement of the lipid radical, the resulting conjugated dienes react with O_2 to form a peroxyl radical (ROO·). Besides damaging nearby intramembraneous protein receptors and enzymes, ROO· also can abstract a hydrogen atom from another fatty acid side chain; thus the process of lipid peroxidation is self-perpetuating (Fig. 4). As the process continues many membrane lipids are converted to lipid peroxides (35). Lipid peroxides within the membrane have a devastating effect on the functional state of the membrane because they alter its fluidity, typically decreasing it and thereby allowing ions such as Ca^{2+} to leak into the cell. Thus lipid peroxidation of cell membranes leads to a chain reaction of events that produce peroxyl radicals and lipid peroxides, both of which are highly destructive to macromolecules and disruptive to key neuronal functions.

Iron and copper play dual roles in lipid peroxidation, both of which have negative consequences. As already noted, both of these ions catalyze the formation of initiating species, e.g., ·OH (Fig. 3). Iron and copper also react with lipid hydroperoxides and decompose them to peroxyl and alkoxyl radicals, which can also reinitiate lipid peroxidation (35). Toxic carbonyl compounds are common products of the complex

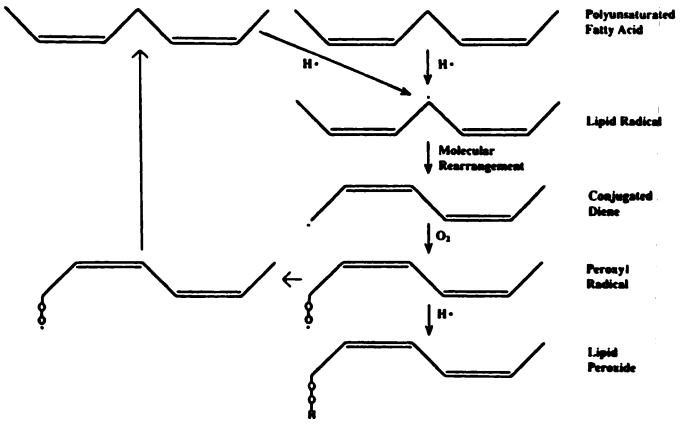

Figure 4. Lipid (polyunsaturated fatty acid) peroxidation. Many initiating radicals remove a hydrogen atom (H·, equivalent to a hydrogen radical) from a fatty acid, eventually resulting in the generation of a peroxyl radical which itself that can feed back and initiate damage to another polyunsaturated fatty acid by removing an H·. Lipid peroxidation can be extensive after a single initiating event because resultant radicals, via the chain reaction described, can reinitiate lipid peroxidation, so the process is self-propagating. This chain reaction can persist long after the original initiating event is passed. The conjugated diene adds an O_2 to produce the peroxyl radical. The peroxyl radical is reduced by the addition of a hydrogen atom to form a lipid peroxide.

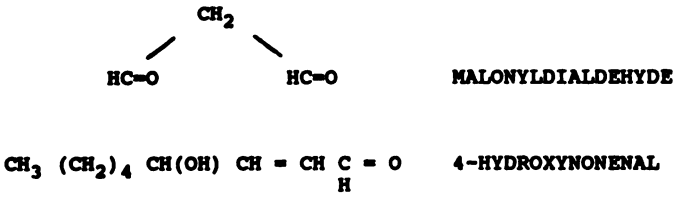

Figure 5. Two products that are often measured as an index of lipid peroxidation; they are often referred to as thiobarbaturic acid reactive substances (TBARS).

processes of lipid decomposition by radicals. Some of these carbonyls are aldehydes including malondialdehyde (MDA, also referred to as malonaldehyde) and 4-hydroxynonenal (**Fig. 5**); other carbonyl products include the highly toxic unsaturated aldehydes such as 4-hydroxyl-2,3-*trans*-nonenal (36) (**Table 2**).

Hydroxyl radicals also attack amino acid residues in protein molecules; these reactions induce extensive protein-protein cross-linking (37). Oxidative damage to proteins by ·OH is magnified by the fact that iron, often associated with proteins, generates the radical in the immediate vicinity of the protein. Once generated, the ·OH readily interacts because of their close proximity with the protein molecule. This is referred to as site-specific damage and it is diagrammatically summarized in **Fig. 6** (6). Damaged proteins, many of them crucial enzymes in neurons, lose their efficiency and cellular function wanes. Protein oxidation in many tissues, including the brain, has been proposed as an explanation for functional deficits associated with aging (37).

Endothelium-derived relaxing factor, or nitric oxide (NO·), contains an unpaired e^- and readily participates in free radical processes that may have pathological significance (38). Besides its production in endothelial cells, NO· is produced in neurons by a calmodulin-activated enzyme, nitric oxide synthase (NOS), which oxidizes arginine to citrulline and requires biopterin, NADPH, and O_2 (39). NO· reacts with the O_2·⁻ to yield a nonradical species, peroxynitrite ($ONOO^-$) (**Fig. 7**) (40). $ONOO^-$ may be directly cytotoxic possibly by oxidizing thiol groups, and there is also evidence that it may decompose to form ·OH (41). There is considerable agreement that the interaction of NO· and O_2·⁻ in neurons is related to normal metabolism as well as to neurodegenerative processes (42). The NO·-generating enzyme NOS is distributed widely in neural tissue.

Collectively, the oxidative processes summarized here provide neural tissue with a continual onslaught of potentially damaging free radicals. Despite a number of molecular defenders (summarized later) against these persistent attacks, some damage, which may or may not be repaired,

TABLE 2 *Aldehyde products resulting from lipid peroidation*[a]

4-Hydroxy Alkenals	2-Alkenals	*n*-Alkenals	Others
4-OH nonenal	Acrolein	Propanal	Malonaldehyde
4-OH hexenal	Pentenal	Butanal	Butanone
4-OH 2,5 monadienal	Hexenal	Pentanal	2,4-Decadienal
	Octenal	Hexanal	
	Nonenal	Nonanal	

[a] In nerve cell membranes these products, some of which are highly toxic, can reek havoc by altering the structure of protein-receptor molecules and destroying enzymes within the membranes.

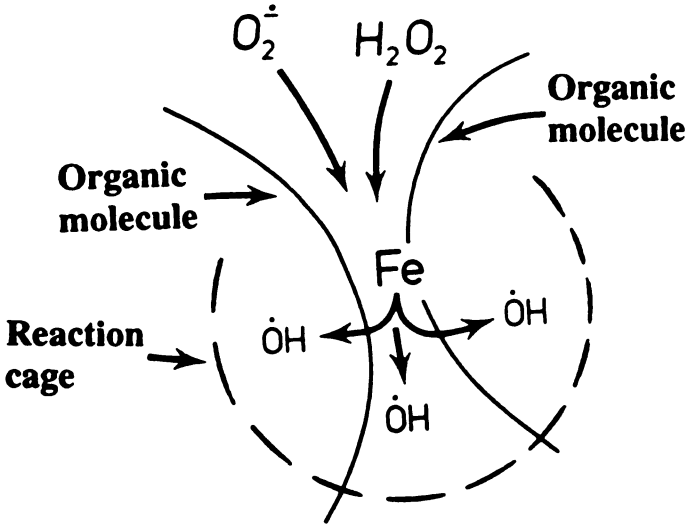

Figure 6. Iron (Fe), bound to organic molecules such as protein, may promote the generation of the highly damaging hydroxyl radical (·OH). The damage inflicted by such radicals is near the site where they are produced; this damage is referred to as being site-specific and occurs within a small area referred to as the reaction cage, which here is demarcated by the dashed line.

results. During aging these damaged molecules, especially in nonreplicating neurons, accumulate. The degree of accumulation depends of how thoroughly free radicals are defended against and the rapidity with which these molecular brigands are produced.

ANTIOXIDATIVE DEFENSE MECHANISMS

Antioxidative enzymes are a primary means by which neural tissue protects itself from toxic oxygen-based radicals. Certainly, SOD activity is a major defense mechanism that combats oxygen toxicity (28, 43) (Fig. 3). SOD, which consists of several isoforms, greatly accelerates the conversion of $O_2^{\cdot}$ to H_2O_2, thereby negating the direct toxic effects of the radical as well as preventing its interaction with metal irons that would culminate in increased ·OH generation (43). The family of SOD enzymes are metalloproteins, and in human tissues the maganese-containing SOD is present in mitochondria whereas the copper- and zinc-rich forms are located in the cytosol. SOD activity is found throughout the central nervous system, with copper/zinc SOD being the major contributor to the total dismutating activity of brain (44).

The efficacy of SOD as an antioxidant relies on its cooperation with other enzymes, i.e., CAT and GSH-Px, which metabolize the dismutation product of $O_2^{\cdot}$, H_2O_2 (Fig. 3). CAT quickly decomposes H_2O_2 to water and O_2, but there is very little CAT activity in the brain so essentially it is inconsequential in this tissue. On the other hand, GSH-Px (a selenium-dependent enzyme) probably plays a major role in disposing of H_2O_2 in neural tissue (45). In this reaction, H_2O_2 is used to oxidize reduced glutathione (a tripeptide, glutamyl-cysteinyl-glycine) (46). Oxidized glutathione, GSSG, is recycled by glutathione reductase (GSH-Rd) (Fig. 3). Both GSH-Px and GSH-Rd are distributed throughout the brain; although GSH-Px usually is considered to be primarily localized in glial cytoplasm, rather than in neurons, some immunocytochemical evidence claims weak GSH-Px activity in neurons as well (47). An extensive review of the literature related to age-associated changes in antioxidant enzymes has recently appeared (9). The conclusion of this survey is that, in neural tissue, the activity of GSH-Px in mammals changes little even in very advanced age. Considering the general lack of change of antioxidative defense mechanisms in the aging brain, Matsuo (9) poses the possibility that there are potent antioxidants yet to be discovered.

Although there are data about the use of synthetic antioxidants, e.g., diprenyl (a MAO-B inhibitor), desferrioxamine (a chelating agent), and novel steroids (21-aminosteroids, lazaroids, and 2-methylaminochromans), in preserving brain morphology and function, information about naturally occurring scavengers that either enter the brain or are produced in neurons is more sparse.

Vitamin E, often represented by α-tocopherol, is a lipid-soluble antioxidant concentrated in the hydrophobic interior of cell membranes. It is known to contribute an e^- to the peroxyl radical that is formed during the chain reaction of lipid peroxidation, and therefore is classified as a chain-breaking antioxidant. The vitamin E radical produced when the parent compound donates an e^- is unreactive, and it eventually degrades or is recycled to vitamin E by ascorbate. The ability of vitamin E to be recycled is an important aspect of its antioxidant activity.

α-Tocopherol is important for normal brain physiology as evidenced by observations that patients with a prolonged deficiency of this vitamin due to intestinal fat absorption problems suffer from neurological deficits (48). Likewise, in cultured neurons vitamin E promotes survival and neurite outgrowth, suggesting that it may be beneficial to these cells in vivo (49). Because of these basic studies using α-tocopherol, a preliminary study of the use of a vitamin E/vitamin C combination therapy for Parkinson's disease has been undertaken (50), but the results are not yet available.

The function of vitamin C (ascorbic acid) in the brain is a double-edged sword with respect to free radical damage. In general, both the gray and white matter of the brain contain substantial amounts of ascorbic acid. Because of a special active transport system in the choroid plexus, ascorbic acid concentrations in the CSF can exceed those in the blood by 10-fold (51). Also, a second transport system is present in neural cells to further concentrate ascorbic acid (51). To identify vitamin C as exclusively an antioxidant is somewhat of an oxymoron. Whereas ascorbate is a free radical

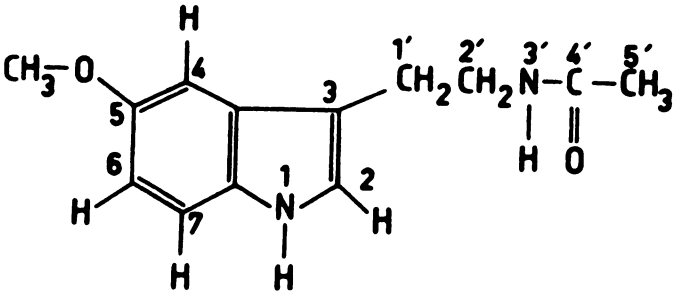

Figure 7. Melatonin, or *N*-acetyl-5-methoxytryptamine, was recently shown to be a protent antioxidant and free radical scavenger. Structure-activity studies have shown that for melatonin to be a hydroxyl radical scavenger; the methoxy group at position 5 of the indole nucleus is a requirement whereas the acetyl group on the side chain has a synergistic action in the scavenging of the ·OH.

scavenger and functions as an antioxidant in recycling the vitamin E radical back to vitamin E, especially in the brain, vitamin C can also be a strong prooxidant. Injecting iron salts into the brain or a rise in the iron content of neuronal tissue after intracerebral hemorrhage leads to increased neural damage due to lipid peroxidation. Increased free radical generation in this case likely is a consequence of the interactions of ascorbate with iron (52). Thus, ascorbic acid is either an antioxidant or a prooxidant in the brain depending on its concentrations and on the availability of other constituents with which it can interact.

Recently it was shown that a constituent produced in the pineal gland, i.e., *N*-acetyl-5-methoxytryptamine or melatonin (Fig. 7), readily passes through the blood-brain barrier (53) and enters neurons and glial cells (54, 55) where it may exert strong antioxidant activity (56, 57). Exogenously administered melatonin quickly enters the brain, and because of its lipid and water solubility (58), it probably distributes readily to all subcellular compartments. This lack of compartmentalization makes melatonin unique among antioxidants that are characteristically confined to one cellular compartment, e.g., vitamin E in lipid cell membranes, vitamin C in the aqueous environment of the cytosol, etc. Preliminary studies indicate that melatonin is an efficient scavenger of both the ·OH (56) and ROO· (57). This indole has been shown to be an efficient protector of nuclear DNA (59, 60), proteins (61), and lipids in cellular membranes (62, 63) against free radical attack. The findings published to date indicate that melatonin restricts lipid peroxidation by preventing the initiating events as well as interrupting the chain reaction. This is the only known antioxidant to have these dual effects. Also, the product that results when melatonin donates an e^- to ·OH is the relatively unreactive indolyl cation radical, which then presumably scavenges a $O_2^{\cdot-}$, thereby further reducing the likelihood of the formation of the highly toxic ·OH (64). Besides its direct scavenging ability, melatonin stimulates brain GSH-Px activity (**Fig. 8**) (65). GSH-Px is perhaps the most important antioxidative enzyme in the brain (45).

Finally, considering the possible role of NO· in damaging free radical reactions (40, 41), that melatonin inhibits NOS activity may be a significant aspect of its antioxidative activity (66). Although the great preponderance of evidence suggests that melatonin is a potent antioxidant, some in vitro data suggest that, at low concentrations, it may also promote free radical production (67). An independent confirmation of this finding is needed.

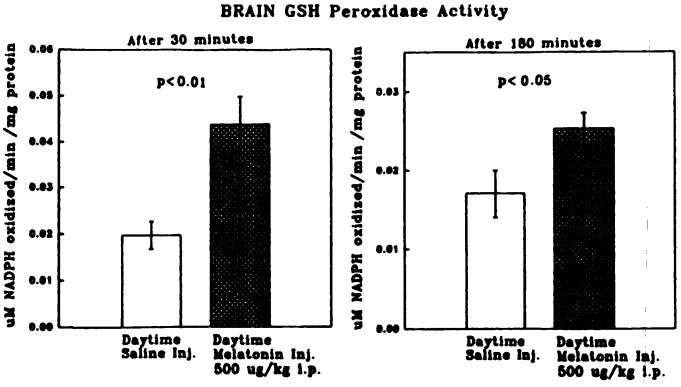

Figure 8. Rat brain glutathione peroxidase activity 30 and 180 min after either an injection of saline or a pharmacological dose of melatonin. Glutathione peroxidase is a major antioxidative enzyme in the brain of mammals. Melatonin readily crosses the blood-brain barrier.

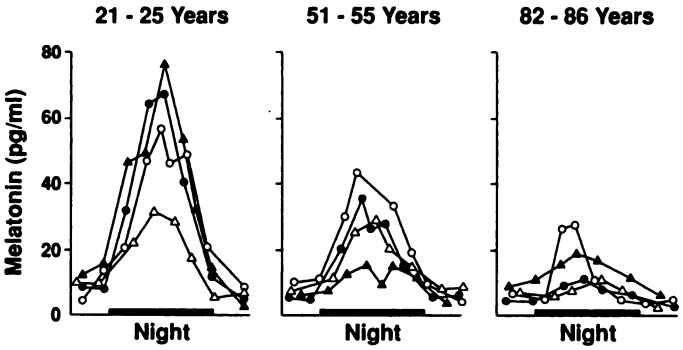

Figure 9. Day and nighttime melatonin levels in four individuals in each age group. Generally, as humans age the nighttime rise in melatonin becomes progressively more attenuated. Also, the amplitude of the nocturnal rise in melatonin varies greatly among individuals of the same age.

On the basis of melatonin's apparently potent antioxidative actions, it may be a significant factor in reducing damage to the brain that is a consequence of oxidative stress (4, 8, 11). Furthermore, it may be significant in brain aging. The production of melatonin falls markedly in advanced age (**Fig. 9**) (68), thereby leaving the organism deprived of one of its seemingly most potent antioxidative defense mechanisms. Indeed, it has been speculated that development of age-related neurodegenerative conditions that involve free radical destruction of cellular organelles and neurons themselves may relate, in part, to the gradual loss of melatonin in advanced age (4, 11, 69, 70). A corollary of this would be that supplemental melatonin during aging may forestall some age-related neurodegenerative diseases, as has been proposed for deprenyl and vitamin E.

CONCLUDING REMARKS

Deterioration of neural function with age is related in many cases to oxidative damage to specific neural areas. Destruction of neurons during aging may be widespread, e.g., after global ischemia, but it is often localized to certain neuronal groups, e.g., at sites where there is a high density of excitatory neuron terminals and the dopaminergic neurons in the substantia nigra, etc. The possibility of deferring age-related degenerative changes in the brain has become a major interest of both experimentalists and clinicians. A number of synthetic molecules that have antioxidative activity are being tested, e.g., deprenyl, for their ability to alter the degenerative processes of the aging brain. To date, none has shown great promise although the clinical tests are still in preliminary stages.

Remarkably, as pointed out earlier, the brain is not unduly enriched with antioxidative defense systems as one might suspect considering its importance to survival. However, there may be a specific reason that the brain is not well protected from damaging oxygen radicals. Once organisms perform the penultimate physiological event, i.e., reproduction, they become expendable to the population. Indeed, early death in these postreproductive animals is important to prevent them from using valuable nutritional resources that should be made available to young animals who have yet to reproduce. Thus, perhaps a poorly developed antioxidative

defense system evolved in the brain to ensure an early death after the organism had reproduced itself. This may be the first example of planned obsolescence. Undoubtedly death of the brain is always associated with death of the individual. It may, however, be within our capability to defer loss of brain function and thereby postpone death (71) with agents that forestall the degeneration and death of the cells in central nervous system. Specifically, what if any natural or synthetic antioxidants will best meet this hope is still the goal of future research. One process that delays aging and death and also reduces oxidative stress is caloric restriction (72). Limited food availability also significantly preserves the pineal production of melatonin (73), potentially a key factor in delaying oxidative age-related neuronal destruction (4, 8, 11). Like underfeeding, providing supplemental melatonin also reportedly maintains animals in a healthy state into advanced age and increases longevity by roughly 20% (74). Experiments designed to confirm this potentially important action of melatonin should have a high priority. FJ

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JULY 1995 THEMATIC ISSUE

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N. Mackman. Transcriptional regulation of the tissue factor gene
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T. F. Tedder. Vascular adhesion molecules, the selectins