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# OXIDATIVE DAMAGE IN THE CENTRAL NERVOUS SYSTEM: PROTECTION BY MELATONIN

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**Abstract**—Melatonin was recently reported to be an effective free radical scavenger and antioxidant. Melatonin is believed to scavenge the highly toxic hydroxyl radical, the peroxynitrite anion, and possibly the peroxyl radical. Also, secondarily, it reportedly scavenges the superoxide anion radical and it quenches singlet oxygen. Additionally, it stimulates mRNA levels for superoxide dismutase and the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (all of which are antioxidative enzymes), thereby increasing its antioxidative capacity. Also, melatonin, at least at some sites, inhibits nitric oxide synthase, a pro-oxidative enzyme. In both *in vivo* and *in vitro* experiments melatonin has been shown to reduce lipid peroxidation and oxidative damage to nuclear DNA. While these effects have been observed primarily using pharmacological doses of melatonin, in a small number of experiments melatonin has been found to be physiologically relevant as an antioxidant as well. The efficacy of melatonin in inhibiting oxidative damage has been tested in a variety of neurological disease models where free radicals have been implicated as being in part causative of the condition. Thus, melatonin has been shown prophylactically to reduce amyloid $\beta$ protein toxicity of Alzheimer's disease, to reduce oxidative damage in several models of Parkinson's disease (dopamine auto-oxidation, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine), to protect against glutamate excitotoxicity, to reduce ischemia-reperfusion injury, to lower neural damage due to $\delta$-aminolevulinic acid (phorphyria), hyperbaric hyperoxia and a variety of neural toxins. Since endogenous melatonin levels fal l markedly in advanced age, the implication of these findings is that the loss of this antioxidant may contribute to the incidence or severity of some age-associated neurodegenerative diseases. 

## CONTENTS



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## ABBREVIATIONS

| | |
|---|---|
| AAPH | 2,2′-Azo-bis(2-amidinopropane) dihydrochloride |
| A$\beta$ | Amyloid beta peptide |
| AD | Alzheimer's disease |
| AIP | Acute intermittent porphyrin |
| ALA | $\delta$-Aminolevulinic acid |
| CAT | Catalase |
| DA | Dopamine |
| DHBA | Dihydroxybenzoic acid |
| DMPO | 5,5-Dimethylpyrroline *N*-oxide |
| $e^-$ | Electron |
| GPx | Glutathione peroxidase |
| GRd | Glutathione reductase |
| G-6PD | Glucose-6-phosphate dehydrogenase |
| GSH | Reduced glutathione |
| GSSG | Oxidized glutathione |
| $H^+$ | Hydrogen atom |
| $HO_2^{\cdot}$ | Hydroperoxy radical |
| $H_2O$ | Water |
| $H_2O_2$ | Hydrogen peroxide |
| KA | Kainic acid |
| $LOO^{\cdot}$ | Peroxyl radical |
| LPS | Lipopolysaccharide |
| MPTP | 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| NAS | *N*-Acetylserotonin |
| NMDA | *N*-methyl-D-aspartate |
| $NO^{\cdot}$ | Nitric oxide |
| $O_2$ | Oxygen |
| $^1O_2$ | Singlet oxygen |
| $O_2^{-\cdot}$ | Superoxide anion radical |
| $^{\cdot}OH$ | Hydroxyl radical |
| 6-OHDA | 6-Hydroxydopamine |
| $ONOO^-$ | Peroxynitrite anion |
| PBN | $\alpha$-Phenylnitrone |
| PD | Parkinson's disease |
| PUFA | Polyunsaturated fatty acid |
| SOD | Superoxide dismutase |
| THA | Terephthalic acid |

## 1. INTRODUCTION

The central nervous system (CNS) is highly susceptible to damage by a variety of biological agents. This problem is enhanced by the fact that neurons and neuron-derived cells, with few exceptions, do not renew themselves so a gradual reduction in these essential elements throughout a lifetime is unavoidable. The insidious reduction in the number of neurons and their synaptic connections eventually compromises virtually all CNS functions. Because of this, a prudent action would be to minimize neuronal loss and thereby possibly forestall the associated neurophysiological and neurobehavioral decrements that are seemingly inevitable in the aged. This would be of particular importance under the current circumstances where improvements in medical sciences have ensured progressively greater longevity in succeeding generations of humans thereby rapidly increasing the number of individuals reaching advanced age in many well developed countries of the world.

Some of the most destructive processes are a direct consequence of one of the inescapable aspects of the environment under which most species live. Thus, an atmosphere consisting of 20% oxygen ($O_2$, dioxygen) is highly destructive because of the oxidizing potential of this and related molecules. These molecules, characterized as reactive oxygen species (ROS) or intermediates, over the course of a lifetime bludgeon essential molecules into submission such that they can no longer function in their normal capacity (Esterbauer, 1985; Stadtman, 1992; Wallace, 1992; Beckman and Ames, 1997). Thus, whereas aerobic organisms cannot obviously survive in the absence of $O_2$, likewise its use in many cases leads to their slow demise. This is known as the oxygen paradox (Halliwell and Gutteridge, 1984) and the destructive properties of $O_2$ and ROS may account for many aspects of aging *per se* (Harman, 1980, 1991; Poeggeler *et al*., 1993; Reiter, 1997a) as well as a variety of medical conditions experienced during senescence (Freeman and Crapo, 1982; Kehrer, 1993; Klaunig *et al*., 1997), including many age-associated, debilitating diseases of the CNS (Beal, 1995; Reiter, 1995a; Hurn *et al*., 1996; Hensley *et al*., 1997).

To survive in an oxidizing environment, which most species do, they must be equipped with the necessary molecular tools to combat at least some of the damaging effects of the $O_2$-rich environment. Fortunately, organisms possess (Sies, 1993; Sies and Stahl, 1995; Vatassery, 1997) or have at their disposal (Jaskot *et al*., 1983; Griffith, 1985; Meister, 1988) molecules, referred to as antioxidants, which help them to resist oxidation. This complex array of processes, identified as the antioxidative defense system, is more or less adequate to counterbalance the multiple oxidative processes in young organisms; however, with advanced age or when organisms are exposed to toxins and/or free radical generating agents the antioxidative defenses may not be up to the task and, as a result, related diseases and signs of aging occur (Fig. 1). Thus, the secret to resisting free radical associated diseases and premature aging may in part depend on the ability of the organisms to cope with and withstand the molecular mutilation that occurs as a consequence of oxidative processes throughout life (Bandy and Davison, 1990; Harman, 1992; Levine and Stadtman, 1992; Reiter *et al*., 1996a).

Of all the organs in the body, the CNS takes more than its share of oxidative abuse (Halliwell and Gutteridge, 1985; Braughler and Hall, 1989; Floyd, 1990). The reasons for this are several-fold. The brain although constituting only a small percentage (in the human about 2%) of the body weight consumes a disproportionately large amount (in the human about 20%) of the $O_2$ inhaled. Given that by-products of $O_2$ are toxic, it is not surprising that neural tissue may thus be destroyed at a more rapid rate than other organs.

Considering its high utilization of $O_2$, one might predict the brain would be endowed with an extra complement of antioxidative processes. Not only does this not seem to be the case, it actually is rather deficient in its ability to resist oxidative plundering. For example, it has low levels of the important anti-

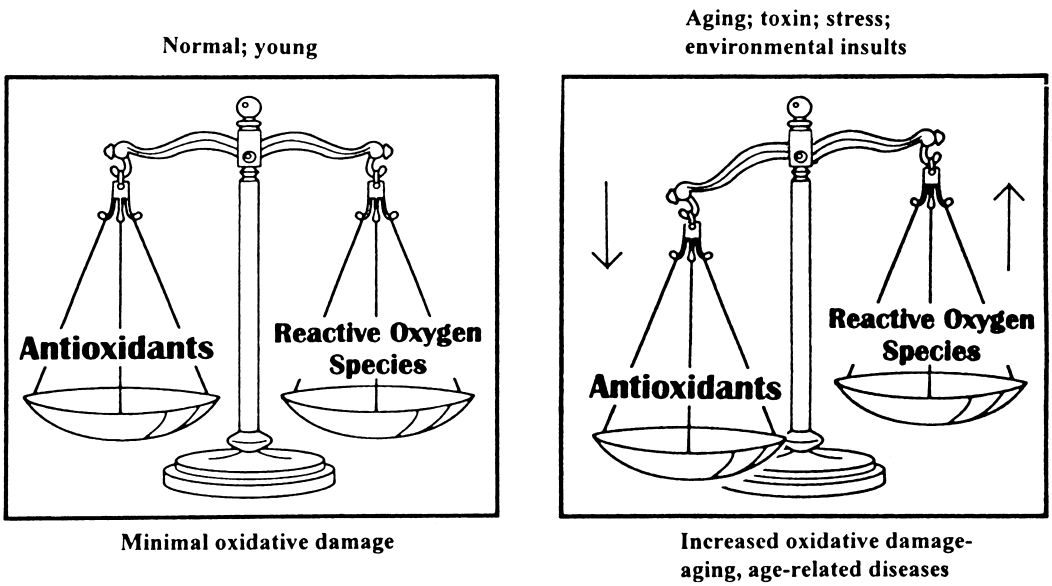


Fig. 1. Diagrammatic representation of the potential role of radicals in aging and the development of age-related diseases that involve free radicals. When organisms are young, free radical generation is more-or-less balanced by a variety of antioxidants. With advancing age and exposure to toxins, stress and environmental insults free radical generation increases and overwhelms the antioxidant defense systems and aging accelerates and age-related diseases develop.

oxidative enzymes (Savolainen, 1978; Bondy, 1997). The brain also contains, at least regionally, high concentrations of iron and ascorbic acid (vitamin C). Unbound iron alone and in combination with ascorbic acid actively generates oxidants (Sadrzadeh *et al.*, 1987). The brain contains high concentrations of polyunsaturated fatty acids (PUFA) in which oxidative processes can be rather easily initiated and once underway the process is self-propagating (Sevanian and McLeod, 1997). The brain is equipped with a morphophysiological barrier, the so-called blood–brain barrier, which fortunately limits the access of many toxins into the CNS but, at the same time, it restricts the entrance of a number of antioxidants. Finally, as noted already, when neurons are lost, the loss is permanent since these cells are differentiated to the point where they can no longer undergo cell division.

## 2. REACTIVE OXYGEN SPECIES AND THEIR TOXICITY

Well over 90% of the $O_2$ that enters human cells is used for the production of energy by mitochondrial cytochrome oxidase; during this process, four electrons ($e^-$) are added to each $O_2$ molecule resulting in the formation of two molecules of water as follows:

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O \qquad (1)$$

An estimated 1–4% of the $O_2$ taken into cells, however, forms partially reduced $O_2$ species, the ROS; some of these contain an unpaired electron and are therefore referred to as free radicals. In the strictest sense the word, 'free' preceding 'radical' is not necessary since all radials are 'free' (Leigh, 1990). In the biological sciences, however, it has become conventional to include the word 'free' to describe a radical and, thus, in the present report the term 'radical' and 'free radical' will be used interchangeably.

The diatomic oxygen molecule, that is, $O_2$, itself qualifies as a radical inasmuch as it possesses two unpaired electrons, each located in a different orbital but both having the same spin quantum number. Because of the parallel spin of these unpaired electrons, $O_2$ itself has relatively low reactivity in contrast to other radicals which can be highly reactive. For $O_2$ to oxidize a molecule directly it would have to accept a pair of electrons, and these electrons would have to have spins opposite to those of the unpaired electrons in $O_2$. The two electrons forming a covalent bond in a molecule would not meet this criterion since they have spins opposite to one another. Thus, the reaction of $O_2$ with non-radical species, which constitutes most molecules in cells, is severely limited by this factor of spin restriction.

### 2.1. Superoxide Anion Radical

Within biological systems, $O_2$ usually accepts electrons one at a time resulting in the following intermediates:

$$\begin{aligned} O_2 &\longrightarrow^{e^-} \text{superoxide anion radical}\left(O_2^{-\cdot}\right) \\ &\longrightarrow^{e^-} \text{hydrogen peroxide}(H_2O_2) \\ &\longrightarrow^{e^-} \text{hydroxyl radical}(^{\cdot}OH) \longrightarrow^{e^-} \text{water}\left(H_2O\right) \end{aligned} \qquad (2)$$

The intermediates have various degrees of reactivity with non-radial species. The acceptance of a single electron by $O_2$ generates the $O_2^{-\cdot}$. The $O_2^{-\cdot}$ is produced *in vivo* in a variety of ways (Fig. 2). The major source of this reactant is via the electron

chain in mitochondria (Nohl and Hegner, 1978; Sohal, 1997). It is generally held that $O_2^{-\cdot}$ is not highly reactive towards biological substrates in an aqueous environment. Moreover, once formed, $O_2^{-\cdot}$ quickly undergoes dismutation to generate $H_2O_2$; this reaction is markedly accelerated by a family of enzymes, the superoxide dismutates (SOD) (Fridovich, 1989). Since SOD removes an oxidant, that is, $O_2^{-\cdot}$, from the cell it is generally considered an important antioxidative enzyme (Touati, 1989).

In solution, $O_2^{-\cdot}$ actually exists in equilibrium with the hydroperoxyl radical ($HO_2^{\cdot}$)

$$O_2^{-\cdot} + H^+ \sim HO_2^{\cdot} \quad (3)$$

Under conditions of tissue acidosis, which can occur in the nervous system during ischemia, equation (3) favors the formation of the $HO_2^{\cdot}$.

$HO_2^{\cdot}$ is a much more lipid soluble and is a far more powerful oxidizing or reducing agent than is $O_2^{-\cdot}$. Thus, in an acidic environment lipid peroxidation, due to the conversion of $O_2^{-\cdot}$ to $HO_2^{\cdot}$, is greater. Finally, $HO_2^{\cdot}$ has a much higher rate of dismutation to $H_2O_2$ than does $O_2^{-\cdot}$.

### 2.2. Hydrogen Peroxide

Besides SOD, several other enzymes that generate $H_2O_2$ also exist in human tissues; these include L-amino acid oxidase, glycolate oxidase and monoamine oxidase. In dopaminergic nerve terminals the oxidative deamination of dopamine (DA) by monoamine oxidase is the chief catabolic pathway for this monoamine (Cohen, 1988). Indeed, it has been proposed that the accelerated turnover of DA in the brain of Parkinson's patients may account for the increase oxidative stress in dopaminergic terminals which eventually leads to their destruction. Likewise, the side effects of prolonged L-DOPA treatment in Parkinson's patients may also relate to excessive $H_2O_2$ formation and its conversion to more highly toxic molecules (Olanow, 1990).

$H_2O_2$ itself is not especially toxic unless it is in high concentrations within cells. There are features of the molecule, however, which make it hazardous. $H_2O_2$ readily diffuses through cellular membranes and can thereby distribute to sites distant from where it was generated. Also, in the presence of transition metals, most often $Fe^{2+}$ (Fig. 2) but also $Cu^{1+}$, $H_2O_2$ is reduced to the hydroxyl radical ($^{\cdot}OH$) via either the Haber–Weiss or Fenton reactions (Imlay *et al.*, 1988; Halliwell and Gutteridge, 1990); Yamasaki and Piette, 1991).

The ultimate fate of $H_2O_2$, however, is not always the $^{\cdot}OH$. In most cells $H_2O_2$ is converted to innocuous products by the actions of two important antioxidative enzymes, that is, catalase and selenium-dependent glutathione peroxidases (GPx) (Fig. 2). In the brain the GPx are considerably more important than catalase because of the low activity of the latter enzyme in most parts of the CNS (Jain *et al.*, 1991). GPx utilizes $H_2O_2$ and hydroperoxides as substrates during the conversion of reduced glutathione (GSH) to its disulfide (GSSG) (Griffith, 1985).

### 2.3. Hydroxyl Radical

The $^{\cdot}OH$ is perhaps not the only destructive species that is formed during the Fenton reaction (Bielski, 1991) but its formation is well documented as is its ability to oxidize adjacent molecules (Halliwell and Gutteridge, 1989). An intermediate in the reaction of $H_2O_2$ with $Fe^{2+}$ may be the iron–oxygen complex referred to as ferryl which itself is

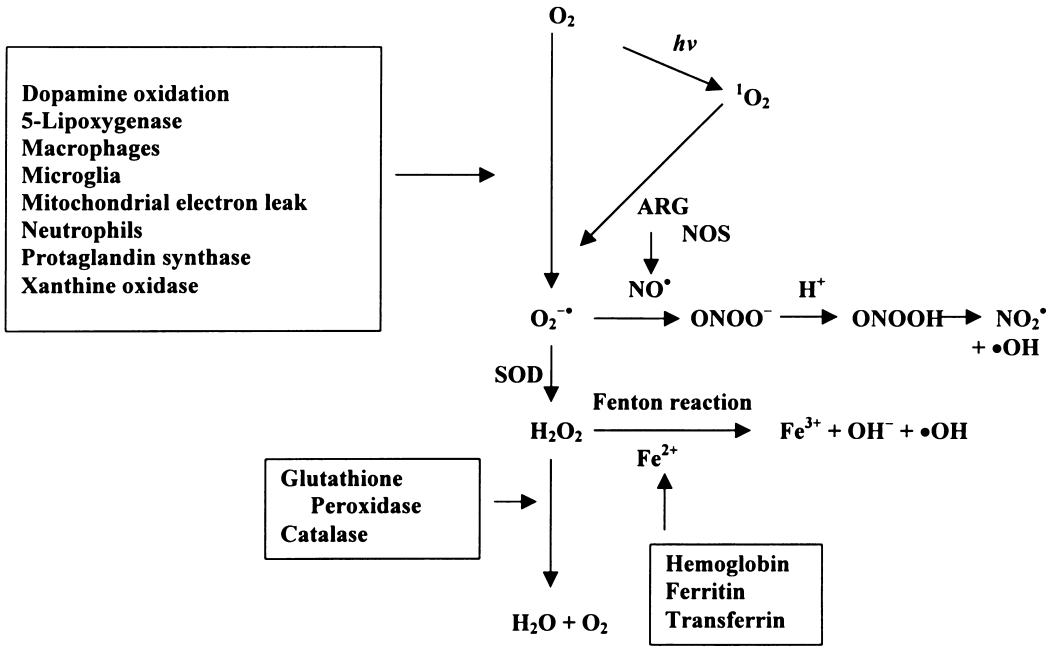


Fig. 2. A summary of the multiple by-products generated by the partial reduction of oxygen ($O_2$). The explanatory details for the pathways, diagrammatically represented here, are summarized in the text.

highly oxidizing and which degrades to form the $^{\cdot}OH$.

There is universal agreement that once formed, the $^{\cdot}OH$ reacts rapidly with any molecule within a few Ångstroms of where it is produced. Because of its high reactivity its estimated half-life at 37°C is on the order of $1 \times 10^{-9}$ sec. The $^{\cdot}OH$ readily damages nuclear and mitochondria DNA, membrane lipids and carbohydrates. When the $^{\cdot}OH$ is produced within mammalian cells it virtually always leaves in its wake damaged DNA products (Cochrane, 1991). There are at least two ways in which DNA damage is achieved. In many cases the mutilated DNA may occur because $H_2O_2$ reacts with either $Fe^{2+}$ or $Cu^{1+}$ that is bound to molecules in the immediate vicinity of DNA so when the toxic $^{\cdot}OH$ is formed its first target is the adjacent nucleic acids (Halliwell and Arouma, 1989). Alternatively, during excitatory neurotransmitter stimulation of neurons, the large increases in intracellular free $Ca^{2+}$ activates nuclease enzymes in the nucleus which results in the formation of $^{\cdot}OH$ which subsequently leads to DNA damage (Orrenius *et al.*, 1989).

Besides its destructive actions at the level of the genetic material in the nucleus, the $^{\cdot}OH$ does not reserve itself specifically for this action. Thus, this semi-reduced oxygen species also interacts with membrane lipids to initiate lipid peroxidation; this is accomplished when the $^{\cdot}OH$ removes an allelic $H^+$ from a PUFA; this results in a radical chain reaction wherein lipid peroxidation is self-propagating (Fig. 3). The process of lipid decomposition is favored during ischemic acidosis. During neural ischemia for example, lipid peroxidation could actually be initiated in the absence of an oxygen radical initiator when iron released from storage proteins reacts with lipid hydroperoxides thereby decomposing them to peroxyl and alkyl radicals which can abstract a $H^+$ leading to further lipid breakdown (Halliwell and Gutteridge, 1990). As already mentioned, the brain is a favorite site for lipid peroxidation because of its regionally high content of iron and due to the fact that neural membrane phospholipids are composed of a high content of easily oxidized PUFA such as linoleic (18:2) and arachidonic acid (20:4).

### 2.4. Singlet Oxygen

Singlet oxygen ($^1O_2$) is formed under photoxidative conditions when energy is transferred to $O_2$ from photoexcited sensitizers, for example, rose bengal or methylene blue (Fig. 2). $^1O_2$ is not a diradical like molecular oxygen and it is highly reactive toward most olefins; thus, it can abstract a $H^+$ from a PUFA to initiate lipid peroxidation. In biological systems, $^1O_2$ may play a prominent role in the peroxidation of membrane lipids (Halliwell and Gutteridge, 1989).

### 2.5. Peroxyl Radical

Perhaps the most thoroughly studied of all oxidative processes is that of the break down of lipids in cellular membranes during which the peroxyl radical is formed (Asano *et al.*, 1991). The process, referred to as lipid peroxidation, is extremely complex and can be self-propagating which means that once initiated it would theoretically lead to the oxidation of all the lipids in a cell; thus, it can be highly destructive. The initiation of lipid peroxidation by the $^{\cdot}OH$ was already summarized above (see Section 2.3). However, the $^{\cdot}OH$ is not the only radical that can begin the process of lipid destruction; $^1O_2$ and $ONOO^-$ can also do so (Sevanian and McLeod, 1997).

Once underway, a number of toxic products are generated during the decomposition of fatty acids. These include, as noted already, lipid hydroperoxides and the peroxyl radical ($LOO^{\cdot}$). The $LOO^{\cdot}$ can then attack a nearby PUFA and re-initiate (propagate) the process (Fig. 3). Vitamin E (α-tocopherol) is the premier $LOO^{\cdot}$ scavenger and chain breaking antioxidant (Packer, 1994).

### 2.6. Nitric Oxide and Peroxynitrite Anion

$NO^{\cdot}$ is often characterized as a double-edged sword. Under normal physiological conditions this nitrogen-centered radical has important functions as a neuronal messenger molecule; however, when $NO^{\cdot}$ increases intracellularly to unusually high concentrations it initiates a toxic cascade of events which can lead to the death of neurons (Dawson *et al.*, 1992; Zhang *et al.*, 1994). A common example of $NO^{\cdot}$ toxicity is seen in glutamate neurotransmission in the CNS where *N*-methyl-D-aspartate (NMDA)-receptor activation leads to large rises in $[Ca^{2+}]_i$ followed by the stimulation of neuronal NOS (Dawson *et al.*, 1991) leading to the generation of $NO^{\cdot}$. As seen in Fig. 4, this induces a series of events that can lead to neuronal destruction.

During focal ischemic events in the CNS the release of excitatory amino acid neurotransmitters including glutamate causes large increases in $NO^{\cdot}$ (Malinski *et al.*, 1993). $NO^{\cdot}$ then interacts with $O_2^{-\cdot}$ to generate the peroxynitrite anion ($ONOO^-$) (Radi *et al.*, 1991a). It is this latter molecule which accounts for much of the toxicity of $NO^{\cdot}$. Hastening $O_2^{-\cdot}$ removal with exaggerated SOD activity reduces infarct volume following focal cerebral ischemia in mice (Kinouchi *et al.*, 1991) as does inhibition of NOS (Dawson and Snyder, 1994). These findings

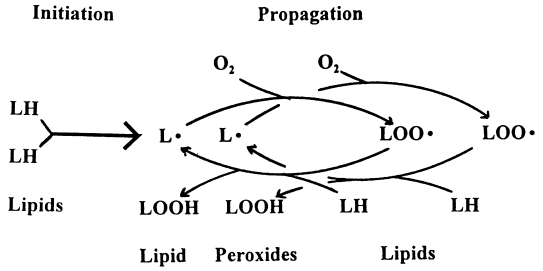


Fig. 3. Lipid peroxidation is an extremely damaging process because once it is initiated it is self sustaining since the peroxyl radical ($LOO^{\cdot}$) can re-initiate (propagate) the process. Theoretically once underway lipid peroxidation, because of this re-initiating chain of events, could go on indefinitely.

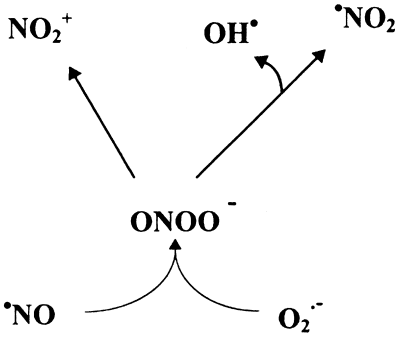


Fig. 4. Nitric oxide $(NO)^{\cdot}$ rapidly combines with the superoxide anion radical $(O_2^{-\cdot})$ to produce a highly toxic agent the peroxynitrite anion $ONOO^{-}$. The molecule can either directly damage lipids, proteins and DNA, or it can do so after it degrades into other highly toxic molecules, that is, the hydroxyl radical ($^{\cdot}OH$) or one that is similarly toxic.

are consistent with $ONOO^{-}$ being the culprit in $NO^{\cdot}$ toxicity (Fig. 4).

Whereas $ONOO^{-}$ is a simple molecule, it is chemically highly complex. Its reactivity is roughly the same as that of $^{\cdot}OH$ and $NO_2^{\cdot}$ (hydroperoxyl radical or hydrogen dioxide). The toxicity of $ONOO^{-}$ derives from its ability to directly nitrate and hydroxylate the aromatic rings of amino acid residues (Beckman *et al.*, 1992), to react with sulphydryls (Radi *et al.*, 1991a), with zinc-thiolate moieties (Crow *et al.*, 1995) as well as with lipids (Radi *et al.*, 1991b), proteins (Moreno and Pryor, 1992) and DNA (King *et al.*, 1992) (Fig. 4). This ubiquitous activity makes the $ONOO^{-}$ a molecule that can have devastating effects on neuronal physiology as well as viability.

## 3. MELATONIN AS A FREE RADICAL SCAVENGER

The toxicity of free radicals can be mitigated by direct free radical scavengers and by indirect antioxidants (Sies, 1993). Examples of molecules that directly scavenge radicals include the tocopherols (vitamin E), ascorbic acid (vitamin C), $\beta$-carotene, and GSH (Halliwell, 1994; Sies and Stahl, 1995). A number of enzymes act as indirect antioxidants since they metabolize free radicals or their reactive intermediates to harmless products. Enzymes which function in this capacity include SOD, GPx, glutathione reductase (GRd) and catalase (CAT).

In the last several years, the chief secretory product of the pineal gland, melatonin (Reiter, 1991), has been found to be both a direct free radical scavenger and an indirect antioxidant (Hardeland *et al.*, 1995; Reiter *et al.*, 1995, 1997a,b; Hardeland, 1997). Because of these actions, melatonin has been pharmacologically tested for its ability to reduce oxidative damage is a variety of experimental neurological processes and it has generally been found highly effective in this regard (Reiter *et al.*, 1997c, 1998a).

Melatonin (*N*-acetyl-5-methoxytryptamine) is a product of tryptophan metabolism and is produced in a limited number of organs in mammals including the pineal gland, retinas and in the gastrointestinal tract (Reiter, 1991). Melatonin has a molecular weight of 232 and is both lipid (Costa *et al.*, 1995, 1997) and water soluble (Shida *et al.*, 1994), although its solubility in lipid is clearly greater. The bulk of the melatonin measured in the blood is derived from the pineal gland and in all mammalian species where it has been measured, blood melatonin concentrations are 5–15 times higher at night than during the day. Melatonin is, however, not exclusively a mammalian synthetic product. It is also found in all non-mammalian vertebrates (Binkley, 1988), in invertebrates (Vivien-Roels and Pevet, 1993) including algae (Poeggeler *et al.*, 1991) and bacteria (Manchester *et al.*, 1995; Tilden *et al.*, 1997) as well as in a variety of edible plants (Hattori *et al.*, 1995; Murch *et al.*, 1997). Thus, melatonin is not only an endogenously produced antioxidant but it can also be ingested. The role of either of these sources in terms of the total antioxidative capacity of the organism has yet to be determined.

The bioavailability of melatonin following its exogenous administration to three mammalian species (rat, dog and monkey) was recently investigated (Yeleswaram *et al.*, 1997). The apparent elimination half-lives following an intravenous dose of either 3 mg $kg^{-1}$ (dog and monkey) or 5 mg $kg^{-1}$ (rat) was 19.8, 18.6 and 34.2 min for the rat, dog and monkey, respectively. In rats, an intraperitoneally (i.p.) administered dose of 10 mg $kg^{-1}$ had a bioavailability of 74% suggesting a lack of an appreciable first pass hepatic extraction in this species; in dogs given melatonin orally, however, bioavailability was reduced to roughly 17%. This suggests marked species differences in the hepatic extraction of melatonin. Studies using a human adenocarcinoma cell line or Coca-2 cells suggest that melatonin would be readily absorbed in the human and there would be a low first-pass hepatic extraction. These observations are consistent with the high concentrations of melatonin in the blood of humans given the indoleamine orally (Aldhous *et al.*, 1985).

As indicated earlier, when injected into animals or given orally, melatonin levels quickly increase in the blood and, followed shortly thereafter, by its uptake into tissues (Menendez-Pelaez *et al.*, 1993; Menendez-Pelaez and Reiter, 1993). Its levels in tissues can exceed blood concentrations manifold. There are no morphophysiological barriers to melatonin; this is apparent in reference to the brain were melatonin concentrations increase soon after the peripheral administration of the indoleamine (Menendez-Pelaez *et al.*, 1993).

Intracellularly, melatonin may not be distributed uniformly. When pharmacological doses of melatonin were given to rats, highest concentrations of melatonin occurred in the nuclei of cells (Menendez-Pelaez *et al.*, 1993). This was determined immunocytochemically and by radioimmunoassay measurement after cells were disrupted and organelles separated by centrifugation. More recently, Finnochiaro and Glikin (1998) examined the subcellular distribution of fluorescein-immunolabeled melatonin in several mouse tissues including NB41A3 neuroblastoma cells. Regardless of the cellular type examined, the workers detected melatonin in both

the cytosol and nuclei. Of special interest was that the apparent quantities of melatonin in the cytosol and nuclei fluctuated with the stage of the cell cycle. On the basis of their findings, Finnochiaro and Glikin (1998) speculated that melatonin has a variety of functions within cells and assumed that one action may be the protection of macromolecules from free radical damage. Additionally, they suggested similar functions of melatonin in all cells.

The first suggestion that melatonin may function as a free radical scavenger came from the work of Ianas *et al.* (1991). Unfortunately, because of the poor quality of the English presentation, it is difficult to determine precisely how effective melatonin was as a free radical scavenger in this *in vitro* study; they did conclude, however, that melatonin possesses both antioxidant and pro-oxidant activity, a feature common to a number of so-called antioxidants. Two years later, Tan *et al.* (1993a,b) provided strong evidence that melatonin was highly effective in detoxifying the highly reactive $^{\cdot}OH$.

### 3.1. Interactions of Melatonin with the Superoxide Anion Radical

The $O_2^{-\cdot}$ which is generated primarily during oxidative phosphorylation in mitochondria (Nohl and Hegner, 1978; Sohal, 1997) and also via a number of enzymatic processes within cells (Halliwell and Gutteridge, 1989) (Fig. 2), is itself not considered highly reactive in aqueous media and it is quickly metabolized primarily by its dismutation to $H_2O_2$ (Fridovich, 1989). $O_2^{-\cdot}$ acts as a moderate reducing agent, for example,in the reduction of cytochrome *c*. $O_2^{-\cdot}$, however, can react with some targets very quickly and when it does so with $NO^{\cdot}$ it generates the $ONOO^-$ (Saran *et al.*, 1990):

$$O_2^{-\cdot} + NO^{\cdot} \longrightarrow ONOO^- \qquad (4)$$

This reaction is directly applicable to the CNS since $NO^{\cdot}$ is produced *in vivo* in many brain cells and in the vascular endothelium of nervous tissue (Moncada *et al.*, 1991). $NO^{\cdot}$ performs a variety of useful functions including the regulation of vascular tone and functioning as a neurotransmitter. Since $NO^{\cdot}$ produces relaxation of smooth muscle cells in blood vessel walls, the interaction of $O_2^{-\cdot}$ with $NO^{\cdot}$ [equation (4)] may lead to vasoconstriction which could have deleterious effects (Nakazono *et al.*, 1991). Of interest is old literature which indicates that pinealectomy (reduction in circulating melatonin) leads to a gradual increase in blood pressure in rats (Karppanen, 1974) which is reversed by melatonin administration (Holmes and Sugden, 1976) This suggests that melatonin's direct or indirect interactions with $NO^{\cdot}$ may be involved in the hypertensive response induced by surgical removal of the pineal gland. This interpretation is complicated by more recent observations which show that melatonin causes vasoconstriction of some vessels by acting on specific receptors in these tissues (Krause *et al.*, 1995); this suggests that pinealectomy and the resulting reduction in melatonin would lead to the relaxation of blood vessels and a reduction in blood pressure.

While the reactivity of $O_2^{-\cdot}$ in mammalian cells is usually considered to be low, there is evidence that in isolated submitochondrial particles $O_2^{-\cdot}$ may inactive the NADH dehydrogenase complex of the mitochondrial electron transport chain (Zhang *et al.*, 1990). Certainly, the protonated form of $O_2^{-\cdot}$, that is, $HO_2^{\cdot}$; is more reactive than $O_2^{-\cdot}$, at least *in vitro*. Thus, while $O_2^{-\cdot}$ is not capable of initiating lipid peroxidation and decomposing lipid hydroperoxides, $HO_2^{\cdot}$ does so (Aikens and Dix, 1991). However, there is no evidence *in vivo* that $HO_2^{\cdot}$ has these effects. At physiological pH values, only a small fraction of $O_2^{-\cdot}$ is believed to be in its protonated form.

Melatonin seems to have little ability to directly scavenge the $O_2^{-\cdot}$. While melatonin's precursor, serotonin, exhibits some reactivity toward $O_2^{-\cdot}$, melatonin does not (Chan and Tang, 1996). These findings are consistent with those of Marshall *et al.* (1996) who also reported that melatonin does not directly react with $O_2^{-\cdot}$ generated by the hypoxanthine–xanthine oxidase system. Whether melatonin reacts with $HO_2^{\cdot}$ has yet to be studied.

There are ways in which melatonin may, however, influence the intracellular concentration of $O_2^{-\cdot}$. As will be discussed next, the product that is formed, that is, the indolyl cation radical (Hardeland *et al.*, 1993), when melatonin donates an electron to a highly reactive free radical such as the $^{\cdot}OH$, is believed to secondarily scavenge $O_2^{-\cdot}$ thereby reducing its levels within cells. Furthermore, melatonin has been reported to increase mRNA levels for SODs (Antolin *et al.*, 1996; Kotler *et al.*, 1998), the family of enzymes that play a major role in the dismutation of $O_2^{-\cdot}$. If the rise in SOD mRNA is associated with a proportional increase in the activity of this family of metalloenzymes, then melatonin would serve to lower intracellular concentrations of $O_2^{-\cdot}$ by stimulating its conversion to $H_2O_2$.

### 3.2. Interactions of Melatonin with Hydrogen Peroxide

Like $O_2^{-\cdot}$, $H_2O_2$ at low micromolar concentrations intracellularly is generally considered to be poorly reactive although higher levels can interfere with energy-producing systems within cells, for example, by reducing the activity of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Cochrane, 1991). In general, the toxicity of $H_2O_2$, as well as that of $O_2^{-\cdot}$, is usually assumed to be a consequence of its conversion to the $^{\cdot}OH$ by the Fenton and Haber–Weiss reactions (Halliwell, 1978). Melatonin has been reported to have no direct reactivity toward $H_2O_2$ in the peroxidase–guaiacol assay (Poeggeler *et al.*, 1994; Tang *et al.*, 1996).

Cells are endowed with enzymes which convert $H_2O_2$ to non-toxic products thereby reducing the formation of the $^{\cdot}OH$. The enzymes which function in this capacity are CAT and GPx (Chance *et al.*, 1979). CAT activity within the brain is considered to have minimal influence as an antioxidative process because of its low activity. On the other hand, the GPx, which require selenium for their action, play an important antioxidant role because they remove $H_2O_2$ when they oxidize GSH to its disulfide

form (GSSG). The activity of GPx is stimulated by exogenously administered melatonin in several organs including the brain (Barlow-Walden *et al*., 1995; Pablos *et al*., 1995a,b, 1997a). Thus, melatonin would be expected to reduce both intracellular $H_2O_2$ levels and ·OH generation. The stimulatory effect of melatonin on GPx is consistent with its rapid uptake into the CNS after its peripheral administration (Menendez-Pelaez *et al*., 1993; Menendez-Pelaez and Reiter, 1993). The potential physiological relevance of melatonin in stimulating GPx activity was recently emphasized by the finding that in several regions of chick brain, GPx exhibits a marked nighttime increase which is prevented when the birds are exposed to constant light (Pablos *et al*., 1998). Since nighttime light exposure prevents the nocturnal production of melatonin, the implication of these findings is that the nightly rise in neural GPx activity may be driven by the associated increase in melatonin (Drijfhout *et al*., 1996).

Once GSH is oxidized to GSSG, this product is regenerated back to GSH in a reaction catalyzed by the flavoprotein enzyme GRd, another important antioxidative enzyme (Fig. 5). Its activity, like that of GPx, is stimulated by exogenously administered melatonin (Pablos *et al*., 1997a) and its nocturnal increase in the chick brain may be a consequence of the nighttime rise in melatonin production (Pablos *et al*., 1998). Finally, a co-factor required for GRd activity, that is, NADPH, is generated by the activity of the enzyme glucose-6-phosphate dehydrogenase (G-6PD) (Fig. 5). According to Pierrefiche and Laborit (1995), G-6PD is also stimulated by melatonin. If these results are validated in a variety of species, collectively these findings will show that melatonin has a significant role in removing $H_2O_2$ from cells by modulating the activity of at least three antioxidative enzymes which directly or indirectly function in the metabolism of $H_2O_2$ to nontoxic products thereby protecting cells from oxidative stress by reducing ·OH generation.

Recent preliminary studies suggest that melatonin may also stimulate the $H_2O_2$ metabolizing enzyme, CAT, in the brain (Montilla *et al*., 1997). If so, the significance of this finding remains unknown since CAT activity in the CNS is generally considered to play a minor role in $H_2O_2$ removal and antioxidative defense.

### 3.3. Interactions of Melatonin with the Hydroxyl Radical

Tan *et al*. (1993a) specifically tested melatonin's ability to scavenge the ·OH. Using a well defined cell free system in which $H_2O_2$ was exposed to ultraviolet light (254 nm) to generate the ·OH, they determined that melatonin competed very effectively with the spin-trapping agent 5,5-dimethylpyrroline *N*-oxide (DMPO) in neutralizing the highly toxic ·OH. When both melatonin and DMPO were present in the mixture, melatonin in increasing concentrations reduced the number of DMPO–·OH adducts which were quantified by HPLC with electrochemical detection and confirmed by electron spin resonance (ESR) spectroscopy. In the same report, Tan *et al*. (1993a) compared melatonin to two well-known ·OH scavengers, that is, glutathione and mannitol, and found that melatonin was significantly better than both these agents. Structure–activity studies revealed that the acetyl group on the side chain and the methoxy group at position 5 of the indole nucleus were both important for melatonin's ·OH scavenging activity.

Using a somewhat different system where ·OH were generated via the Fenton reaction in the presence of DMPO and melatonin, Matuszak *et al*.

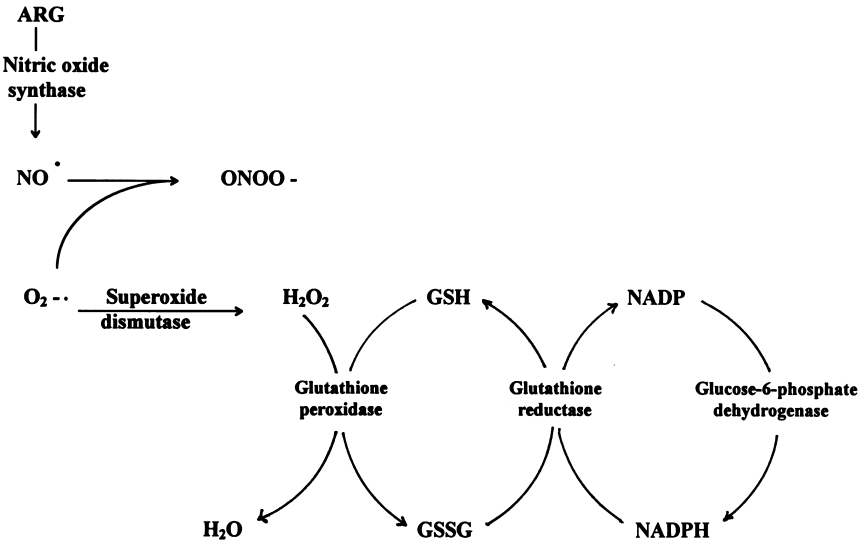


Fig. 5. Diagrammatic representation of the fates of $O_2^{-}\cdot$; it can combine with nitric oxide to generate the peroxynitrite anion ($ONOO^{-}$) or it is dismutated to hydrogen peroxide ($H_2O_2$). $H_2O_2$ is utilized as a substrate by GPx when it oxidized glutathione (GSH) to its disulfide (GSSG); the latter molecule is reduced back to GSH by GRd which requires the co-factor NADPH. GPx, GRd and G-6PD have been shown to be stimulated by melatonin. This would remove $H_2O_2$ from cells and reduce the likelihood of it being converted to the hydroxyl radical.

(1997) also reported the indoleamine to be a highly effective ·OH scavenger. Like Tan *et al.* (1993a), they used ESR spectroscopy to verify their findings and calculated that the rate constant for the scavenging of the ·OH by melatonin was $2.7 \times 10^{10}$ $M^{-1}$ $sec^{-1}$.

Also using ESR spectroscopy to identify and quantify the DMPO–·OH, Susa *et al.* (1997) found that melatonin reduced chromium (VI)-related ·OH generation. They further showed that DNA damage typical of that induced by the ·OH was reduced in the presence of melatonin. Their conclusion was that melatonin is a potent free radical scavenger and that it effectively protects against DNA strand breaks, lipid peroxidation and cytotoxicity caused by chromium.

The ·OH radical scavenging activity of melatonin *in vitro* has also been confirmed by Pähkla *et al.* (1998) who used terephthalic acid (THA) as a chemical dosimeter of ·OH since it forms an adduct, that is, THA–·OH (Barreto *et al.*, 1994). In this system, melatonin, in a concentration dependent manner, reduced the formation of the THA–·OH with the $IC_{50}$ for melatonin being $11.4 \pm 1.0$ $\mu$M. This $IC_{50}$ value for melatonin is not greatly different than that reported by Tan *et al.* (1993a) where the $IC_{50}$ for the ability of melatonin to reduce DMPO–·OH formation was 21 $\mu$M.

From simple cell free systems such as those used by Tan *et al.* (1993a,b), Matuszak *et al.* (1997) and Pähkla *et al.* (1998) it is difficult to determine the *in vivo* relevance of melatonin as a physiological ·OH scavenger. Within cells there are numerous molecules that could function in the detoxification of the ·OH and which would compete with melatonin for this function. Since physiologically, melatonin levels within cells seem quite low (in the nanomolar range) and other potential scavengers exceed its concentration by several orders of magnitude, the degree to which endogenous melatonin protects against free radical damage via direct free radical scavenging remains to be determined. Melatonin's action in this regard will only be known when all of its antioxidative actions are determined (they seem to be multiple) and more information is available concerning its concentrations within subcellular compartments. Thus, for the time being it would seem prudent to simply classify melatonin as a pharmacological antioxidant in mammals.

There is, however, one organism (*Gonyaulax polyedra*) in which intracellular melatonin levels are very high and in which these physiological concentrations are sufficient to protect the organism from oxidative damage (Antolin *et al.*, 1997). Furthermore, pinealectomy, which merely reduces endogenous circulating levels of melatonin, exaggerates oxidative damage after treatment of rats with free radical generating agents (Tan *et al.*, 1994; Manev *et al.*, 1996b). Thus, the possibility that physiological levels of melatonin contribute to the total free radical scavenging capacity of the organism should not be totally disregarded at this point.

Poeggeler *et al.* (1994, 1995, 1996) further showed that melatonin is also an efficient radical scavenger in other *in vitro* systems. By measuring changes in indole fluorescence, they showed that melatonin was quickly oxidized by ·OH generated with Fenton reagents but not by iron itself. Likewise, they found that melatonin was oxidized in the presence of $H_2O_2$ only and finally that melatonin synergized with other antioxidants, for example, vitamins C and E, in the scavenging of radicals. This is an important observation because it suggests that *in vivo*, particularly in the presence of other free radical scavengers, melatonin could have a role as a physiologically relevant antioxidant.

In detoxifying the ·OH radical, melatonin is believed to work via electron donation (Hardeland *et al.*, 1993; Poeggeler *et al.*, 1994, 1996). In so doing, melatonin must itself become a radical, the indolyl cation (or melatonyl) radical, of which there may be several isoforms (Stasica *et al.*, 1998)

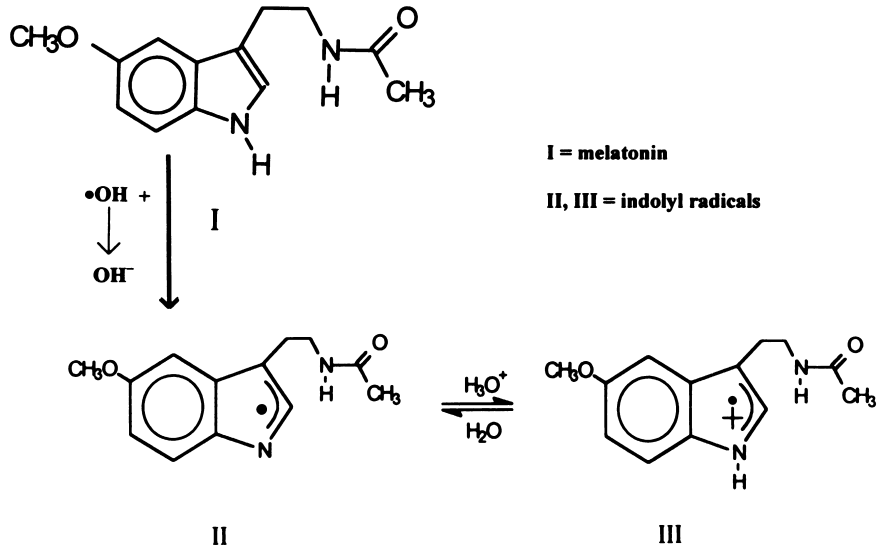


Fig. 6. Melatonin (I) is believed to detoxify highly toxic radicals by electron donation. In doing so, it becomes the indolyl cation radical, of which there may be more than one form (II and III).

(Fig. 6). The indolyl cation radical may then scavenger a $O_2^-$ thereby further reducing the number of $^{\cdot}OH$ generated since $O_2^{-\cdot}$ is its precursor (Hardeland *et al*., 1993; Poeggeler *et al*., 1994). Thus, each melatonin molecule apparently has the capability to scavenge two radicals.

Evidence that exogenously administered melatonin acts *in vivo* to scavenge the $^{\cdot}OH$ was recently provided by Li *et al*. (1997). This group used the salicylate trapping method to show that melatonin administration to rats undergoing ischemia-reperfusion of the brain reduced dihydroxybenzoic acid (DHBA) in the microdialysate retrieved from the ischemic brain. DHBA is a specific product formed by the interaction of the $^{\cdot}OH$ and salicylate and its reduction indicates that melatonin scavenged $^{\cdot}OH$ thereby leading a reduced DHBA formation. This is the first evidence which shows that melatonin functions as a $^{\cdot}OH$ quencher *in vivo*.

### 3.4. Interactions of Melatonin with Singlet Oxygen

The evidence that melatonin detoxifies $^1O_2$ comes from indirect evidence provided by Cagnoli *et al*. (1995). This group showed that the neurotoxic effects of $^1O_2$ *in vitro* were counteracted by melatonin. To achieve this, newborn rat cerebellar granule cells were treated with a photosensitizing dye, rose bengal, and they were then exposed to light, a procedure which generates $^1O_2$ (Agarwal *et al*., 1991). This reactive molecule is believed responsible for cell death during ischemia-reperfusion injury and may play a role in Parkinson's disease (PD) (Perry and Young, 1986). Based on the suggestion of Poeggeler *et al*. (1993) that melatonin may quench $^1O_2$, Cagnoli *et al*. (1995) added melatonin to the culture medium of granule cells treated with rose bengal and exposed to light. Both neuronal apoptosis and inhibition of creatine kinase activity were prevented by the addition of melatonin. Since melatonin in pharmacological concentrations overcame the photodynamic injury to the neurons, the authors surmised that melatonin directly neutralized $^1O_2$; this observation requires more direct evidence before it is certain that melatonin functions as a $^1O_2$ quencher.

### 3.5. Interactions of Melatonin with the Peroxyl Radical

The $LOO^{\cdot}$ is highly devastating not only because of its high reactivity but also due to the fact that it re-initiates (propagates) the process of the oxidation of lipids (Fig. 3) (Cheesman, 1993). Thus, once underway lipid peroxidation becomes a vicious cycle and, theoretically at least, the oxidation of a single PUFA could lead to the breakdown of all such molecules in the cell. Lipid peroxidation is usually defined as the oxidative mutilation of lipids and it usually refers to fatty acid oxidation, although cholesterol can also be a target of this process. Lipid peroxidation is especially damaging in the CNS since this tissue contains high concentrations of easily oxidizable PUFA (Reiter, 1995a). The destruction of membrane lipids compromises the functions of membrane localized receptors and channels as well as the transmembrane passage of solutes and the fluidity of the membrane. Interruption of the process of lipid peroxidation is a necessary and critical function of lipid soluble antioxidants and they can either inhibit the initiation step or interrupt its propagation, that is, perform as a chain breaking antioxidant. As described above, melatonin scavenges several ROSs, for example, $^1O_2$, $ONOO^-$ and the $^{\cdot}OH$, that are capable of initiating lipid peroxidation. A role for melatonin in the detoxification of the $LOO^{\cdot}$ has also been claimed.

The ability of melatonin to detoxify the $LOO^{\cdot}$ was first proposed by Pieri *et al*. (1994, 1995). In their *in vitro* assay they used the peroxyl radical initiator 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) to induce lysis of human erythrocytes and then compared melatonin, vitamin E (Trolox), vitamin C, GSH and mannitol in terms of their ability to resist oxidative damage. Estimates of the hemolysis of a 1% human erythrocyte suspension after a 3 hr incubation with AAPH indicated that melatonin was superior to the other $LOO^{\cdot}$ scavenging in reducing red blood cell damage. In each case the inhibition of erythrocyte lysis was related to the dose of antioxidant and when the efficiencies of the five molecules were compared that of melatonin > vitamin E > vitamin C > GSH > mannitol. Considering that each molecule of vitamin E scavenges two $LOO^{\cdot}$ (Burton *et al*., 1983), Pieri *et al*. (1995) assumed that a single molecule of melatonin detoxifies four $LOO^{\cdot}$, a finding that clearly requires confirmation.

Melatonin's efficiency as a $LOO^{\cdot}$ scavenger has been investigated and questioned by others as well. According to Scaiano (1995) melatonin, in a competitive situation, would be nearly as effective as vitamin E in trapping free radicals (Evans *et al*., 1992); this description of the efficacy of melatonin in scavenging the $LOO^{\cdot}$ differs slightly from that of Pieri *et al*. (1994, 1995) but it still suggests that the indole is a lipid antioxidant. Scaiano (1995) also concluded that both the 5-methoxy group on the indole nucleus as well as the side chain are essential for the free radical scavenging activity of melatonin; this is consistent with the earlier observations of Tan *et al*. (1993a) who came to the same conclusion. Melatonin was also found to scavenge the trichloromethylperoxyl radical with a rate constant of $2.7 \times 10^8\ M^{-1}\ sec^{-1}$ (Marshall *et al*., 1996); this group felt that melatonin may neutralize the $LOO^{\cdot}$, although the specific nature of the interaction remains unclear.

While melatonin has proven effective in a number of tests designed to determine whether it detoxifies the $LOO^{\cdot}$ exactly how effective it is relative to vitamin E continues to be debated. Vitamin E is widely considered to be the premier $LOO^{\cdot}$ scavenger (Liebler, 1993) and the claim by Pieri *et al*. (1994, 1995) that melatonin is superior to vitamin E in this regard requires further documentation. Other workers report melatonin may effectively somehow neutralize the peroxyl radical, but the efficiency with which it does so is roughly equal to or slightly less than that of vitamin E (Scaiano, 1995; Marshall *et al*., 1996). Melatonin is, however, an inhibitor of lipid peroxidation (Reiter *et al*., 1996b, 1997a;

Reiter, 1997a) which could well involve processes other than its direct $LOO^{\cdot}$ scavenging ability.

### 3.6. Interactions of Melatonin with Nitric Oxide and Peroxynitrite Anion

$NO^{\cdot}$, or endothelium derived relaxing factor, is an important second messenger in neurons (Feldman *et al.*, 1993). This gaseous mediator is generally per se not considered to be highly toxic but after it interacts with the $O_2^{-\cdot}$ it forms the $ONOO^-$ which bludgeons a number of macromolecules (Beckman *et al.*, 1990). This being the case, nitric oxide synthase (NOS) could be classified as a pro-oxidative enzyme (Lipton *et al.*, 1993).

It was recently reported that, at physiological concentrations, melatonin inhibits rat cerebellar (Pozo *et al.*, 1994) and hypothalamic NOS activity (Bettahi *et al.*, 1996). In these studies NOS inhibition by melatonin was dose-dependent and coupled to a reduction in cGMP production activated by L-arginine. Additionally, it was shown that melatonin concentrations measured in the blood of chicks inversely correlates with $NO^{\cdot}$ and cGMP levels in the cerebral cortex (Guerrero *et al.*, 1997).

The melatonin-induced suppression of NOS activity is believed to be a consequence of the binding of calmodulin by melatonin (Pozo *et al.*, 1997; Anton-Tay *et al.*, 1998). NOS is a calmodulin-activated enzyme (Bredt and Synder, 1990) and by binding calmodulin melatonin may limit its availability for this function. With a drop of $NO^{\cdot}$ synthesis, the formation of the $ONOO^-$ is curtailed, and the potential oxidative damage resulting from this latter molecule is averted (Pryor and Squadrito, 1995). Whether melatonin reduces NOS activity in all tissues that contain this enzyme is unknown.

Besides reducing $NO^{\cdot}$ formation by restricting the activation of NOS and thereby limiting the secondary cytotoxicity caused by $ONOO^-$, melatonin was recently shown to directly scavenge the latter molecule as well (Gilad *et al.*, 1997). $ONOO^-$ toxicity not only relates to its ability to initiate lipid peroxidation, but it also reduces mitchondrial respiration, inhibits the function of membrane pumps, depletes cellular GSH, and damages DNA leading to activation of poly(ADP ribose) synthase which causes cellular energy depletion (Pryor and Squadrito, 1995; Szabo, 1996). Gilad *et al.* (1997) used the ability of $ONOO^-$ to oxidize dihydrorhodamine 123 as an index of melatonin's efficacy to scavenge the anion. The addition of melatonin to the mixture led to a dose-dependent inhibition of the oxidation of dihydrorhodamine 123 to rhodamine. This group further showed that melatonin also reduced DNA strand beaks in J774 macrophages incubated with $ONOO^-$ and reduced the degree of suppression of mitochondrial respiration. The potency of melatonin to limit $ONOO^-$ damage was comparable to that of other highly effective peroxynitrite scavengers, GSH and cysteine (Gilad *et al.*, 1997). The authors did note, however, that melatonin even at low mM concentrations, could not totally reverse the toxicity of $ONOO^-$ at the level of mitochondrial respiration.

That the *in vitro* studies of Gilad *et al.* (1997) have applicability to the *in vivo* situation was documented by Cuzzocrea *et al.* (1997) who showed that melatonin reduced the inflammatory response induced by carrageenan where both $NO^{\cdot}$ and $ONOO^-$ are believed to mediate the inflammation (Beckman and Koppenol, 1996; Szabo, 1996). In the comprehensive study of Cuzzocrea *et al.* (1997) they demonstrated, using several different endpoints, melatonin's anti-inflammatory ability and theorized this was related to the ability of the indole to inhibit NOS activity and to scavenge $ONOO^-$ and the $^{\cdot}OH$. These findings were extended by the same group (Cuzzocrea *et al.*, 1998) where melatonin was found to potently inhibit the severe inflammatory response in rats following the injection of a non-bacterial proinflammatory molecule, zymosan; this molecule, in addition to initiating a marked inflammatory response, causes multiple organ failure (Mainous *et al.*, 1995). Again, Cuzzocrea *et al.* (1998) speculated that melatonin's protective effects were a consequence of its ability to reduce $NO^{\cdot}$ formation and scavenge $ONOO^-$ and associated oxidants.

### 3.7. Melatonin and Membrane Fluidity

Fluidity is defined as the quality of ease of movement, whereas viscosity is the quality of resistance to movement (Zimmer and Freisleben, 1988); these features are obviously interrelated. A membrane is an anisotropic two-dimensional fluid exhibiting both lateral and rotational mobilities of proteins and lipids. Both proteins and lipid–protein interactions account for membrane fluidity (Zimmer, 1984). Membrane fluidity is a parameter specific for each type of membrane depending on its molecular composition. An optimal fluidity exists for the specific functions of a given membrane and alterations in membrane fluidity change the functional dynamics of the membrane. Under conditions of oxidative stress, membrane fluidity is often decreased (Victor *et al.*, 1985), possibly due to the oxidation of lipid double bonds and amino acid side chains. Owing to its high solubility in lipid, vitamin E has been widely investigated as to its effects on the fluidity of membranes (Urano and Matsuo, 1989).

Like vitamin E, melatonin is also capable of protecting against the reductions in membrane fluidity and associated processes of lipid peroxidation. Using hepatic microsomes, Garcia *et al.* (1997) showed that the reduction in membrane fluidity, estimated with the aid of fluorescence spectroscopic techniques, induced by treatment with a combination of $FeCl_3$, ADP and NADPH, was reversed in a dose-dependent manner by the addition of melatonin to the culture medium. The effects of melatonin correlated with the ability of the indole to inhibit the peroxidation of lipids in the microsomes. The authors surmised that the stabilizing action of melatonin was a consequence of its free radical scavenging activity. Peroxidation of lipids not only increases the rigidity (decreases the fluidity) of microsomal membranes but other cellular membranes as well (Watanabe *et al.*, 1990; Ghosh *et al.*, 1993); thus, it can be safely assumed that melatonin's ability to protect against fluidity changes is not restricted to microsomes but extends to membranes in general. Likewise, although these studies utilized

hepatic microsomes, the information obtained is applicable to other tissues as well, including the brain where melatonin has been shown to inhibit lipid peroxidation (Reiter *et al*., 1997a).

Since melatonin has actions similar to the antiestrogen tamoxifen, including the inhibition of lipid peroxidation and the stabilization of cell membranes, Garcia *et al*. (1998) also studied these agents in combination in terms of their ability to prevent membrane rigidity. While each drug independently partially reduced microsomal membrane fluidity due to treatment with $FeCl_3$, ADP and NADPH, in combination the effect of these agents was greater than the sums of the two individually. The implication of these findings is that, in the clinical situation where tamoxifen, among many beneficial effects, also functions as a genotoxic carcinogen (Hard *et al*., 1993), a therapeutic regimen including melatonin plus tamoxifen may allow for a lower dose of the latter agent thereby reducing its collateral toxicity. Certainly, the ability of melatonin and tamoxifen to cooperatively interact in membrane stabilization further emphasizes the potential importance of melatonin in maintaining cellular membranes in an optimally functional state.

## 4. DISEASE MODELS OF FREE RADICAL DAMAGE IN THE CNS: INFLUENCE OF MELATONIN

As mentioned already, the amount of abuse the brain takes from free radicals is generally considered to be extensive (Halliwell and Gutteridge, 1985; Poeggeler *et al*., 1993; Reiter, 1995a; Reiter *et al*., 1997c). Perhaps the major reasons for this are its high utilization of $O_2$, its relatively poorly developed antioxidant network, and the fact that it contains large amounts of easily oxidizable fatty acids. As a consequence, the molecular carnage and cytotoxicity that is measured in the brain after toxin exposure and during aging is often substantial. This oxidative damage has been considered a common link in the pathogenesis and neuropathology of a variety of neurodegenerative disorders (Table 1).

Undoubtedly, one of the major challenges for contemporary neurology is the deferral and prevention of age-associated neurodegenerative conditions that are commonplace in a population whose life span has shown substantial increases in recent decades. The debilitating consequences of brain deterioration and malfunction obviously compromise the quality of life and longevity and, additionally, they are financially taxing to society. The scientific quest to identify the causes and effective treatments for these devastating conditions is diverse and intensive. While oxidative stress may be one feature that links many neurological deficits, it is also obvious that these diseases have extremely complex etiopathologies and it is unlikely that a single agent will totally combat their development. Melatonin, however, is of interest in this context for several reasons:

1. the endogenous production of this molecule falls dramatically with age (Reiter *et al*., 1980, 1981; Touitou *et al*., 1981; Iguchi *et al*., 1982; Sack *et al*., 1986; Reiter, 1992) coincident with the onset of many of the age-associated neurodegenerative conditions (Beal, 1995; Hurn *et al*., 1996; Hensley *et al*., 1997);
2. melatonin readily crosses the blood–brain barrier and after its exogenous administration it is found in high concentrations in the brain, sometimes exceeding those in the blood manifold (Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez *et al*., 1993; Finnochiaro and Glikin, 1998);
3. melatonin is a ubiquitously acting free radical scavenger and antioxidant (Hardeland *et al*., 1993; Poeggeler *et al*., 1993; Reiter *et al*., 1994; Reiter, 1995b,c) which in models of neurological diseases has proven effective in reducing oxidative damage and preserving neurological function (Reiter, 1995a; Reiter *et al*., 1998a);
4. the only procedure, that is, food restriction, in animal models of aging that significantly delays senescence also retards the age-associated loss of melatonin (Stokkan *et al*., 1991; Henden *et al*., 1992) suggesting a potential association between the loss on melatonin and the signs of aging.

What follows is a summary of neurological disease models where melatonin has been tested as a palliative agent.

### 4.1. Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of progressive cognitive decline in the aged population and affects an estimated 15 million people worldwide with this number expected to increase with the continuing rise in mean longevity. A triad of neuromorphysiological features characterize AD and include amyloid-$\beta$ plaques (senile pla-

Table 1. Neurological diseases and processes which are believed to involve free radicals

| | |
|---|---|
| Alzheimer's disease | Neuronal ceroid-lipofuscinosis (Batten's disease) |
| Amyotrophic lateral sclerosis | Neuronal apoptosis |
| Autoimmunodeficiency syndrome (HIV infection) | Neurotoxin exposure (e.g. MPTP) |
| | Parkinson's disease |
| Down's syndrome | Progeria |
| Epileptic seizures | Schizophrenia |
| Head trauma | Spinal cord injury |
| Hyperbaric hyperoxia | Tardive dyskinesia |
| Inflammation | Werdnig–Hoffman disease |
| Ischemia/reperfusion | Viral infections |
| Muscular dystropies | Vitamin E deficiency |
| Myesthenia gravis | Xenobiotic-induced nerve damage |

ques), neurofibrillary tangles and extensive neural loss particularly in the hippocampus and cerebral cortex (Koh *et al*., 1990; O'Banion *et al*., 1994; Mattson *et al*., 1997); these changes are associated with dementia and characteristic neurobehavioral consequences. The signs of the disease changes in the majority of cases appear to arise sporadically and usually have a late life onset (after 65 years of age) (Harman, 1995). In a less common form of familial AD, the onset of the condition is typically much earlier (40–50 years of age).

That oxidative stress may be a culprit in neuronal loss in AD has been emphasized in recent years and the evidence is becoming progressively stronger that radicals are involved in the neural pathogenesis of this disease (Hensley *et al*., 1995; Richardson and Zhou, 1996; Markesbery, 1997). The free radicals that have been incriminated as causing neuronal loss are believed to be generated by amyloid $\beta$ (A$\beta$)-peptide (Robakis and Pappolla, 1994; Benzi and Noretti, 1995; Harris *et al*., 1995; Manelli and Puttfarcken, 1995). In particular, the 25–35 amino acid residue of the larger A$\beta$ peptide (consisting of 1 to 39–43 peptides) is believed to be especially efficient in generating free radicals which lead to a series of events which destroy adjacent neurons (Kang *et al*., 1987; Butterfield *et al*., 1994) (Fig. 7).

The potential involvement of ROS in neuronal death in AD led Pappolla *et al*. (1997a,b) to investigate the effect of melatonin on the sequence of events leading to neuronal destruction. Using both murine neuroblastoma (N2a) and PC12 (pheochromocytoma) cells, this group demonstrated that co-incubation of these cells with either A$\beta$ peptide(35–45) or A$\beta$ peptide(1–40) and melatonin greatly reduced cellular death compared to that caused by either peptide alone. Furthermore, the degree of A$\beta$-induced lipid peroxidation in the cells was reduced in the presence of melatonin as were the very high intracellular calcium levels that were measured in the cells incubated with only A$\beta$. Finally, cellular shrinkage and the development of membranous bleds, features characteristic of apoptosis, were greatly reduced when melatonin was added to the A$\beta$-containing medium. Given these findings, Pappolla *et al*. (1997a,b) speculated that melatonin, because of its free radical scavenging activity, neutralized the radicals that were generated extracellularly by A$\beta$ peptide as well as those produced intracellularly by the markedly increased calcium; thereby reducing the neurotoxicity of the A$\beta$ peptide leading to increased cellular survival (Fig. 7). The feature that makes the findings of special interest is that melatonin greatly increased the survival of the cells in this study. These observations, coupled with the known reduction in melatonin (Reiter, 1992) in the aged, opens the possibility that the loss of this antioxidant in the later stages of life may predispose individuals to the neurotoxic effects of A$\beta$ peptide and therefore to AD. Antioxidants in general are receiving increased interest as agents that may defer the onset of this devastating disease.

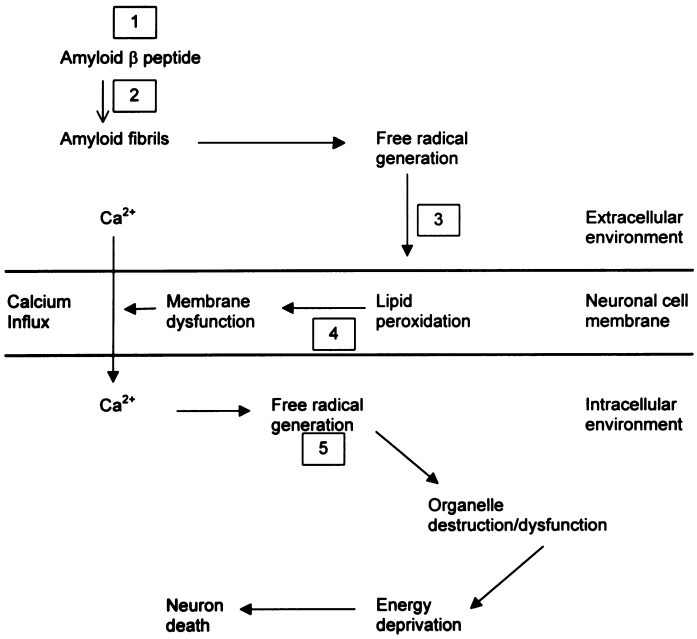


Fig. 7. A proposed mechanism by which amyloid $\beta$ protein generates free radicals and promotes the destruction of neurons in AD. This is sometimes referred to as the shrapnel model of AD. The model includes multiple changes in membrane function including oxidation of lipids, alterations in transport proteins, changes in channel functions, and perturbations of enzyme activities. $Ca^{2+}$ influx leads to high $[Ca^{2+}]_i$ which further generates destructive radicals which can destroy the function of mitochondria which leads to cellular death as a consequence of energy depletion. The numbers 1–5 indicate the sites at which melatonin may act to interfere with A$\beta$ peptide toxicity: 1, preventing the generation of A$\beta$ peptide; 2, reducing the formation of A$\beta$ fibrils; 3, scavenging free radicals generated extracellularly by A$\beta$ fibrils; 4, reducing lipid peroxidation and preventing membrane dysfunction by scavenging the peroxyl radical; 5, scavenging radicals generated intracellularly as a result of increased $[Ca^{2+}]_i$.

Other studies also indicate a potential role for melatonin in delaying the onset of AD. The A$\beta$ peptide in the brain of AD patients is cleaved from the larger glycosalated membrane-bound A$\beta$ precursor protein (A$\beta$PP) (Yoshikawa, 1993a; Selkoe, 1994). Soluble derivatives of A$\beta$PP (sAPP) which lack a cytoplamic tail, transmembrane domain, and a small portion of the extracellular domain are proteolytically generated by a family of secretases. *In vitro* melatonin pharmacologically was found to strongly inhibit the production of sAPP from neuroblastoma and PC12 cells (Song and Lahiri, 1997). This effect of melatonin was reversible. The implication of these findings is that melatonin may reduce the generation of A$\beta$ peptide and, thus, also thereby secondarily reduce neuronal death. In addition, Pappolla *et al.* (1997c, 1998) have recently found that the aggregation of A$\beta$ peptide into $\beta$-sheets and amyloid fibrils, which are important for the toxicity of the peptide and renders it resistance to proteolytic degradation, is greatly reduced by melatonin. Collectively, the findings suggest that melatonin may act in a variety of ways to reduce neuronal loss in AD patients by altering the processes of A$\beta$ peptide generation and action (Fig. 7). The studies of Pappolla *et al.* (1997a,b, 1998), as well as those of Song and Lahiri (1997) were, however, conducted *in vitro* and how the findings relate to the *in vivo* situation remains uninvestigated.

That melatonin reduced lipid peroxidation induced by A$\beta$ peptide was also investigated by Daniels *et al.* (1998). Human platelets incubated with A$\beta$ peptide dose-dependently exhibited increased lipid peroxidation. Melatonin added to the culture medium reduced lipid decomposition that was caused by A$\beta$ peptide. Since aluminum has also been implicated in the etiology of AD (Kawakara *et al.*, 1992), Daniels *et al.* (1998) also tested whether melatonin would influence the toxicity of this metal. Like A$\beta$ peptide, aluminum was found to induce lipid peroxidation with the response being inhibited by melatonin. While these effects of melatonin were speculated to be related to the free radical scavenging and antioxidant activities of melatonin, the response to aluminum could also be a consequence of the binding of this metal ion by melatonin, an observation that was recently reported by Limson *et al.* (1998).

The results summarized suggest that melatonin, for a variety of different reasons, may be beneficial in curtailing some of the damaging neural processes associated with AD. These findings seem to have attracted the attention of experimental neuroscientists and it is likely that additional studies investigating melatonin's actions in neural models of AD will be forthcoming in the near future.

### 4.2. Parkinson's Disease

Parkinson's disease is a major neurodegenerative disorder with a prevalence of roughly 150 cases for every 100 000 elderly people. The condition is characterized by the progressive deterioration of the DA containing neurons in the pars compata of the substantia nigra in the brain stem (Bernheimer *et al.*, 1973; Fearnley and Lees, 1991). The loss of these catecholaminergic neurons is associated with a variety of sensory and motor impairments which lead to tremor, rigidity and akinesia (Ben-Shackar *et al.*, 1986). For an individual to manifest signs of PD it is estimated that the nigro-straital dopaminergic neuronal population must be depleted by at least 80% (Lees, 1992). Thus, in most cases the initiating factor for PD probably precedes the overt signs of Parkinsonism by 5–10 years. As with AD, there are many theories to explain the etiopathology of PD; however, one common denominator of most of these theories is the involvement of free radicals (Olanow, 1990; Fahn and Cohen, 1992; Youdim *et al.*, 1993; Yoshikawa, 1993b).

According to the free radical theory of PD, dopaminergic neurons are lost as a consequence of their relatively high exposure to ROS, most notably $H_2O_2$ which is produced during both the enzymatic, via monoamine oxidase activity, and non-enzymatic, due to the auto-oxidation, destruction of DA (Fahn and Cohen, 1992). Not only does oxidative stress destroy the dopaminergic neurons but it also compromises mitochondrial oxidative phosphorylation leading to decreased energy output by these organelles and eventually to secondary death of the cells. There is ample evidence that the brain of PD patients exhibits signs of enhanced oxidative stress (Perry and Young, 1986; Dexter *et al.*, 1989): Miller *et al.* (1996) assessed the ability of melatonin to protect against DA autoxidation-induced protein damage using the oxygen radical absorbance capacity assay (Cao *et al.*, 1993). Their results showed that melatonin does reduce the degree of oxidation of the fluorescent protein which is the basis for the assay indicating that melatonin prevents macromolecular damage that is a result of DA autoxidation. The authors surmised that this was due to the free radical scavenging capacity of melatonin and they suggested that the indole may have beneficial effects in reducing oxidative damage in the brain of PD patients.

Perhaps the most commonly used model of PD in experimental neuroscience is of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity (Löschmann *et al.*, 1994). This cytotoxin has provided important information on the potential mechanisms of PD. MPTP is taken up by astrocytes where it is metabolized to methyl-4-phenylpyridinium ion (MPP+); this cation is released from the astrocytes and taken up by dopaminergic neurons where it has a variety of actions which lead to cellular death. Presumably included in the actions of MPP+ are its ability to deplete the cells of ATP (Chan *et al.*, 1991), to generate free radicals (Lai *et al.*, 1993) and to induce apoptosis (Dipasquale *et al.*, 1991). When Acuña-Castroviejo *et al.* (1997) used this model of PD to examine melatonin's ability to reduce the effects of MPTP administration into mice, they found that the increased lipid peroxidation that occurred in the straitum, hippocampus and midbrain after MPTP injection was overcome by melatonin co-administration. Immunocytochemically, they also found that melatonin reduced the drop in tyrosine hydroxylase immunoreactivity in the striatum of MPTP-treated mice. The specific effects of MPTP alone or in com-

bination with melatonin on the DA neurons of the substantia nigra were not evaluated in this study.

These preliminary findings were followed by more thorough *in vitro* studies which further suggest the ability of melatonin as a potential anti-Parkinson agent. In the study in question, the ability of melatonin to rescue DA neurons from death in several models where oxidative stress was a major component was examined (Iacovitti *et al*., 1997). In the initial trial, dopaminergic cells, plated at low density, were grown in serum-free media, a procedure that led to death of virtually all the cells within 2–3 days presumably because they were derived of essential growth factors; when melatonin was added to the serum-free medium the majority of the neurons, including the tyrosine hydroxylase immunopositive cells, were maintained for at least 7 days. Similarly, in a second model of oxidative stress, melatonin protected dopaminergic neurons from the neurotoxic injury induced by MPP + . While it is premature to assume that melatonin may be beneficial to patients developing PD, the findings of Acuña-Castroviejo *et al*. (1997) and Iacovitti *et al*. (1997) which used a reasonably well defined model for the disease indicates a clinical trial should be considered.

Another model of PD involves the used of 6-hydroxydopamine (6-OHDA). This agent, like MPTP, destroys catecholaminergic neurons, also via free radical mechanisms (Cohen and Heikkila, 1974; Zigmond and Sticker, 1989), and when injected into the substantia nigra of rats it causes degeneration of the nigro-striatal system (Michel and Hefty, 1990). Using cell mobility, signs of apoptosis, and quantitation of DNA fragmentation, 6-OHDA was found to both damage and kill naïve (undifferentiated) and neuronal (differentiated) PC12 cells in culture when melatonin was absent from the culture medium (Mayo *et al*., 1998a). In the presence of melatonin, the cytotoxicity of 6-OHDA, as indicted by all the measured parameters, was significantly reduced. Since 6-OHDA by itself caused significantly reductions in mRMA levels for three antioxidative enzymes, that is, manganese superoxide dismutase (MnSOD), copper–zinc superoxide dismutase (CuZuSOD) and GPx, and these decrements were prevented by melatonin, the authors judged that the protective actions of melatonin were in part a consequence of the preservation of the activity of these antioxidative catalysts. They also predicted other beneficial actions of melatonin on 6-OHDA-treated cells as well and, in a second report (Mayo *et al*., 1998b), they found that the antiproliferative effects of melatonin may also be involved in protecting at least undifferentiated cells from 6-OHDA toxicity. Thus, the antiapoptotic actions of melatonin in this system are believed to be due to both the antioxidant and antiproliferative effects of the indole (Mayo *et al*., 1998b). Since the 6-OHDA model in animals is a surrogate for PD in humans, the findings of Mayo *et al*. (1998a,b) also suggest the potential utility of melatonin in restraining dopaminergic cell dysfunction and loss in PD.

### 4.3. Excitotoxicity

The excitatory amino acid neurotransmitter glutamate is present in millimolar levels throughout the gray matter of the CNS. These high levels of glutamate are typically concentrated in nerve terminals, and after its release energy-dependent cellular uptake mechanisms quickly clears the released glutamate from the synaptic cleft. As a result, neurons are usually only briefly exposed to the excitatory neurotransmitter and the injury to the adjacent neurons is minimal. Under certain conditions, for example, during episodes of hypoxia or ischemia, reduced cellular reuptake combined with increase glutamate efflux (due to depolarization of glutatmate-containing neurons) can read to large and sustained build-up of the neurotransmitter in the synaptic cleft (Choi, 1988). Also, certain disease conditions involving energy depletion reduce the efficacy of neurons to correct perturbations, for example, increased $[Ca^{2+}]_i$, induced by glutamate exposure (Novelli *et al*., 1988). This combination of disturbances can cause glutamate exposure to become cytotoxic, a phenomenon generally referred to excitotoxicity, with the toxic effects being related to large increases in free radical generation (Schulz *et al*., 1995); this can lead to death of the postsynaptic neurons, where these changes occur (Fig. 8) (Coyle and Puttfarcken, 1993).

Glutamate interacts with a number of receptors after it is released, one of which is the NMDA receptor. Kainic acid (KA) is an agonist of the NMDA receptor (Nakanishi, 1992) and when injected either systemically or intracerebrally into rodents it causes generalized limbic seizures and frequently extensive neuronal damage (Köhler, 1984). These consequences are generally accepted as involving the excessive production of radicals and ROS. In view of this, the KA model of excitotoxicity has been widely used to test the efficacy of free radical scavengers and antioxidants in the CNS.

Melatonin's ability to reduce the toxic effects KA were first investigated by Melchiorri *et al*. (1995). In these *in vitro* studies, melatonin in a dose–response manner reduced KA-induced lipid peroxidation in homogenates of the cerebellum, hippocampus, hypothalamus and striatum of both Wistar and Sprague–Dawley rats; the magnitude of the reduction in of the products of lipid peroxidation varied from 10–100%. In homogenates of whole brains, a similar high degree of protection by melatonin was seen when the damaged lipid products, malondialdehyde and 4-hydroxyalkenals, were used as an index of KA cytotoxicity (Melchiorri *et al*., 1996).

Similar protective actions of melatonin were observed when neuronal cell cultures were utilized in lieu of tissue homogenates. Thus, Giusti *et al*. (1995, 1997) used newborn rat cerebellar granule cell cultures to examine melatonin's antiexcitotoxicity. Like Melchiorri *et al*. (1995), they also found melatonin to be beneficial in preventing KA-induced excitotoxicity and on the basis of their studies they predicted that melatonin's protective actions were related to the radical scavenging properties of the molecule. They drew this conclusion after they showed that melatonin's effect is not related to the

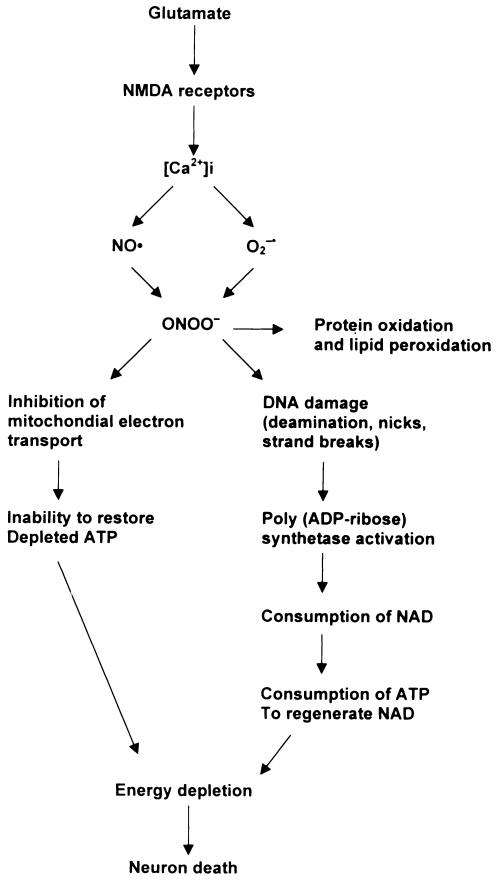


Fig. 8. The action of the excitatory amino acid neurotransmitter, glutamate, on the NMDA receptor causes the influx of calcium into the postsynaptic neuron. This leads to the generation of $NO^{\cdot}$ and $O_2^{-\cdot}$ which combine to form the peroxynitrite anion ($ONOO^-$). This latter molecule causes not only oxidative damage to proteins and lipids but also to DNA bases which fragment leading to the activation of PARS [poly (ADP-ribose) synthetase]. NAD is then used in the ADP-ribosylation of nuclear proteins. For every mole of NAD that is consumed, four free energy equivalents of ATP are required to regenerate NAD. This high utilization of energy coupled to inhibition of oxidative phosphorylation by $ONOO^-$ compromises the cell's capacity to maintain its energy levels. With the energy supply of the neuron depleted, the cell dies.

inhibition of ionotropic glutamate receptors and melatonin did not compete with the binding of glutamate to its receptor on cerebellar granular neurons. Likewise, Lezoualc'h *et al.* (1996, 1998) reported that oxidative apoptotic cell death induced by glutamate in a cultured cloned hippocampal cell line (HT22) and in organotypic hippocampal brain slices was reduced by melatonin. DNA fragmentation typical of programmed cell death, detected by Hoechst staining of the cells, and the degree of damage was also partially reversed by melatonin. Melatonin also reduced the toxicity of $H_2O_2$ to neurons in hippocampal slices and the authors surmised that the antioxidant properties of melatonin afforded the indole its antiexcitotoxic property.

Finally, Cazevieille *et al.* (1997) similarly reported that melatonin was neuroprotective against glutamate and hypoxia/reperfusion mediated by NMDA receptors in rat cortical neurons. Like Giusti *et al.* (1995), they proved melatonin did not have this effect due to an action of the indole at the level of the NMDA receptor. Cazevieille *et al.* (1997) also reported that melatonin and the iron chelator desferoxamine reduced free radical damage in a retinal cell preparation induced by a combination of ascorbate and iron. Their findings, using both corticol and retinal neurons, are consistent with melatonin's neuroprotective actions being related to its functions as a radical scavenger and antioxidant.

Numerous *in vivo* studies have also revealed melatonin's anti-excitotoxic actions; these studies have employed neurobehavioral, biochemical and morphological criteria to demonstrate melatonin's ability to reduce the consequences of KA-mediated excitotoxicity. Using the quantitative TUNEL (dUTP-biotin nick end-labeling) technique and Nissl straining, Manev *et al.* (1996a) found melatonin to reduce KA-triggered DNA damage and cellular death in the CA3 region of the hippocampus, the amygada and the entorhinal cortex. These findings were confirmed by Uz *et al.* (1996) who used the same methods to document neural damage provoked by kainate and reduced by melatonin. Biochemically, melatonin prevented the decrease in noradrenaline and the associated rise in 5-hydroxyindoleacetic acid in limbic structures of rats i.p. injected with KA (Giusti *et al.*, 1996) and also overcame epileptic seizures induced by the excitatory neurotransmitter (Giusti *et al.*, 1996; Manev *et al.*, 1996b).

Besides melatonin's direct scavenging activity its protective effect against KA toxicity may also relate to the ability of the indole to maintain glutathione homeostasis in neurons as shown by Floreani and Lees (1991). When KA was i.p. administered to rats, the drug decreased GSH levels in the amygada and hippocampus but not in the striatum which is resistant to the injury normally inflicted by KA; KA reduced GRd activity in the limbic structures as well. The changes in GSH and GRd were overcome in KA-injected rats co-administered melatonin. The ability of melatonin to maintain glutathione homeostasis would certainly benefit the neurons considering the important role of GSH in reducing oxidative damage. The sparing effect of melatonin on GSH may have been secondary to the direct radical scavenging properties of the indole.

Not only pharmacologically, but physiological levels of melatonin as well have been shown to reduce the excitotoxic activity of KA. When pinealectomized rats (which have chronically low circulating melatonin levels) were treated with KA the neurodegeneration was greater than that observed in similarly-treated intact rats (which exhibit a nightly rise in circulating melatonin levels) (Manev *et al.*, 1996b). The indices of oxidative neural damage in this study included the TUNEL assay for DNA damage and Nissl staining for cell death. The same group showed that hippocampal 5-lipoxygenase mRNA levels are increased in what they refer to as melatonin-deficient, pinealectomized rats (Uz *et al.*, 1997). These findings suggest that the products of 5-lipoxygenase activity, that is, leukotrienes, are toni-

cally reduced by the presence of melatonin synthesized and secreted by the pineal gland. Since leukotrienes are considered to contribute to neurodegeneration (Ohtsuki *et al.*, 1995), the findings of Uz *et al.* (1997) are especially important in the context of the loss of melatonin during aging (Reiter, 1992).

Overwhelmingly, the studies in which melatonin was tested as an anti-excitotoxic agent have yielded positive results. Certainly at pharmacological concentrations, but at physiological levels as well, the pineal-derived secretory product has proven effective in overcoming the neurobehavioral, biochemical, morphological and molecular damage caused by KA. As with other findings summarized in this review, the results are consistent with the idea that melatonin plays a substantial role in neuroprotection.

### 4.4. Porphyric Neuropathy

Acute intermittent porphyrin (AIP) is a dominant inherited metabolic disorder of haem biosynthesis which leads to the urinary excretion of increased amounts of the porphyrin precursors, $\delta$-aminolevulinic acid (ALA) and porphobilinogen (Moore, 1993). Recent data strongly suggest that ROS may account for much of the pathophysiology of AIP since ALA undergoes enolization and subsequent iron catalyzed-oxidation which leads to the generation of $O_2^{-\cdot}$, $H_2O_2$ and the $^{\cdot}OH$. Since ALA accumulates in neural tissues, the neuropathogenesis apparent in AIP patients is believed to be at least partly a secondary consequence of free radical production.

Three reports have been published which illustrate the protective actions of melatonin against ALA toxicity. In rat cerebellar tissue incubated with ALA, lipid damage as measured by the levels of malondialdehyde was highly significantly increased; however, when melatonin was also present in the medium it reduced, in a dose–response manner, the concentrations of damaged lipid products (Princ *et al.*, 1997). Similar observations were made by Carneiro and Reiter (1998) who showed that in homogenates of either cerebral cortex, cerebellum or striatum ALA-toxicity to PUFAs was readily inhibited by melatonin. These authors were of the opinion that melatonin's protective actions were attributable to its free radical scavenging properties.

Both these groups (Carneiro and Reiter, 1998; Princ *et al.*, 1998) also showed that melatonin's neuroprotective actions against ALA extended to the *in vivo* situation as well. Thus, the deleterious effects of ALA in the nervous system, as indicated by the levels of damaged lipid products, were greatly reduced or overcome by melatonin administration.

These findings are of special interest not only because they imply a potential use of melatonin to reduce ALA toxicity in AIP patients, but also because humans suffering with this genetic disorder have reduced levels of circulating melatonin (Puy *et al.*, 1993, 1996), a fact that could aggravate the neurotoxicity actions of ALA. This further emphasizes the potential therapeutic value of melatonin in AIP subjects.

### 4.5. Ischemia-Reperfusion Injury

Like the neuropathological models described, neural stroke also is considered to result in brain damage due at least in part to free radicals that are produced especially during reperfusion (Fig. 9) (Christensen *et al.*, 1994; Wei *et al.*, 1997). There are a variety of animal models in which to study neural injury induced by transient ischemia followed by reperfusion (Krieglstein and Oberpichler-Schwenk, 1994). Only a few of these models have been exploited in studies investigating the effects of melatonin in neuroprotection during ischemia/reperfusion injury.

Cho *et al.* (1997) determined that transient forebrain ischemia in the rat followed by reperfusion caused, 7 days later, a marked loss of CA1 pyramidal neurons in the hippocampus; the period of ischemia was either 10, 20 or 30 min. Melatonin, when given i.p. at the onset of reperfusion or at 2 and 6 hr after reoxygenation, greatly reduced the number of hippocampal neurons lost. One of the candidate mechanisms that the authors proposed for the protective action of melatonin was its antioxidant activity. Cho *et al.* (1997) claimed that melatonin given 30 min prior to reperfusion onset was ineffective in reducing neuronal loss due to ischemia/reperfusion. This is somewhat surprising since in many other models of oxidative neurotoxicity, melatonin given in advance (usually 20–30 min before) of toxin exposure, readily prevented oxidative damage (Reiter, 1997b; Reiter *et al.*, 1997a,b).

The Mongolian gerbil (*Meriones unguiculatus*) has been widely used as a model in which to investigate ischemia/reperfusion brain damage because this species has an incomplete circle of Willis and therefore merely ligating the carotid arteries bilaterally totally interrupts the blood supply to the forebrain. Guerrero *et al.* (1997) used this model in which to examine the potentially beneficial effects of melato-

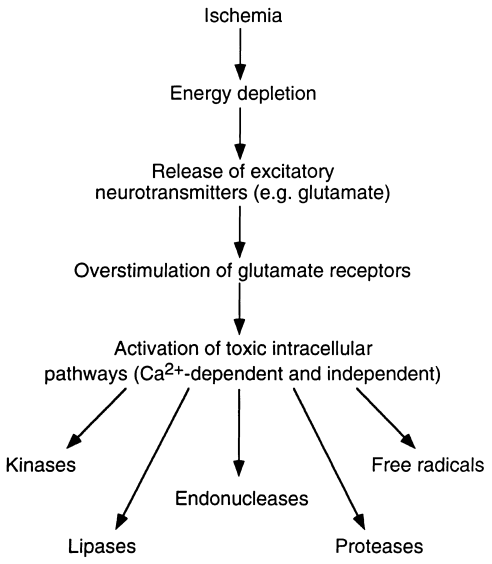


Fig. 9. Ischemia/reperfusion injury, or stroke, in the brain damages and kills many cells via multiple processes. Involved in these processes are free radicals.

nin against an ischemic insult. Since $NO^{\cdot}$ has been implicated as a damaging agent in ischemia/reperfusion injury, the authors measured nitrite/nitrate and cGMP levels as indices of $NO^{\cdot}$ production. A 10 min ischemic period due to bilateral occlusion of the carotid arteries followed by 5 min reperfusion was followed by a rise in cerebral nitrite/nitrate levels and cGMP concentrations. Melatonin, given in pharmacological concentrations prior to interruption of the cerebral blood supply, prevented the changes reported above suggesting that melatonin inhibited $NO^{\cdot}$ production. This would be consistent with the observations of others (Bettahi *et al*., 1996; Pozo *et al*., 1997) who report an inhibitory effect of melatonin an NOS, the enzyme that determines the quantity of $NO^{\cdot}$ produced. The report of Guerrero *et al*. (1997) included no estimates of morphological or physiological brain damage. Escames *et al*. (1997) have shown, however, that melatonin (and vitamin E) also protects brain homogenates from nitric oxide-induced lipid peroxidation *in vitro*; in these studies vitamin E was more efficient in doing so than was melatonin.

Importantly, in addition to the pharmacological studies summarized already (Cho *et al*., 1997; Guerrero *et al*., 1997), Manev *et al*. (1996b) examined the effect of physiological levels of melatonin on neural damage after middle cerebral artery occlusion in the rat. In melatonin-deficient rats due to pinealectomy, infarct volume was greatly exaggerated compared to that in the brain of rats that also suffered middle cerebral artery occlusion but also had an intact pineal gland and, therefore, a normal endogenous melatonin cycle. Again, the observations of Manev *et al*. (1996b) indicate that cerebrovascular accidents in the elderly, who lack a robust melatonin rhythm (Reiter, 1992), may suffer more extensive neural damage than would a young individual who has higher melatonin levels on average.

Besides the brain *per se*, neural retinal cells in culture are protected from experimental ischemia/reoxygenation (8 hr ischemia followed by 16 hr reoxygenation) when melatonin is in the growth medium (Cazevieille and Osbourne, 1997). Since glutamate is released in excess from these cells during ischemia and the resulting damage is believed to be related to free radical production after the interaction of the neurotransmitter with its receptors, Cazevieille and Osbourne (1997) presumed that melatonin preserved retinal neuronal integrity because of its free radical scavenging properties.

Besides its direct protective effects in neural tissue, there may be another reason melatonin helps to preserve brain integrity after ischemia/reperfusion injury. Bertuglia *et al*. (1996) have found that damage to the microvasculature during ischemia/reperfusion is prevented by melatonin. Thus, the increased number of leucocytes adherent to the endothelial cells and the greater edema seen in ischemia tissue are reduced by melatonin application. Since brain edema contributes significantly to the amount of neural tissue damaged, these actions of melatonin may also be essential to the neuroprotective effects of the indole.

### 4.6. Hyperoxia

Oxygen, since it is the source of the majority of free radicals generated in aerobic organs (Halliwell and Gutteridge, 1984), is considered highly toxic. Thus, it is not surprising that the exposure of animals to hyperbaric hyperoxia leads to free radical damage in a number of organs including the brain (Torbati *et al*., 1992). Also, obvious is that since hyperoxia produces its damage due to the generation of radicals, melatonin should afford some protection. This was the rationale of Pablos *et al*. (1997b) when they exposed rats to 100% $O_2$ at 4 atm with and without melatonin pretreatment. Within 90 min, the rats exposed to hyperbaric hyperoxia exhibited significant increases in cerebral cortical, hippocampal, hypothalamic, striatal and cerebellar malondialdehyde and 4-hydroxyalkenals levels, responses that were prevented in rats treated with melatonin prior to the onset of high $O_2$ exposure. Likewise, each of these neural tissues exhibited reductions in the activities of two important antioxidative enzymes, GPx and GRd, after hyperbaric hyperoxia exposure (Pablos *et al*., 1997b). Again, these reductions were overcome in the melatonin-treated rats. These findings are consistent with the oxygen radical scavenging activity of melatonin and the results also emphasize the fact that melatonin quickly crosses the blood–brain barrier and enters cells to protect them from oxidative abuse.

Bovine cerebral endothelial cells grown in culture have been shown to exhibit DNA damage and undergo apoptosis when they are exposed to 95 or 100% oxygen. These responses to hyperoxia are reduced by melatonin in a dose-dependent manner (Shaikh *et al*., 1997). Certainly the partial or total destruction of cerebral endothelial cells by ROS would increase the vulnerability of the brain to damage from a wide variety of toxic agents.

### 4.7. Traumatic Brain Injury

Considering that free radicals play a deleterious role in traumatic brain injury (TBI), Mésenge *et al*. (1998) deemed it important to examine the potential protective role of melatonin in a mouse model; in this study melatonin's efficacy was compared with that of α-phenylnitrone (PBN), a spin trapping agent known to detoxify free radicals. Head injury was induced in mice using a free falling standardized weight as described by Hall *et al*. (1993) and the associated neurological deficit was evaluated 24 hr later by a grip test, which measures the length of time mice remain on a taut string suspended 40 cm in the air. Both PBN and melatonin proved to be neuroprotective as judged by the improved length of time the animals remained on the string relative to TBI mice not given either antioxidant. The total dose of PBN given in this test was 40 times greater than the dose of melatonin suggesting the latter molecule is very protective in protecting against TBI.

### 4.8. Neural Toxins

A dose- and time-dependent increase in products of lipid peroxidation occurs in the brain of mice fol-

lowing the administration of a sublethal dose of potassium cyanide (Johnson *et al.*, 1987). Furthermore, seizures are a consequence of the intoxication of mice with cyanide. Yamamoto and Yang (1996a,b) presumable that some of the neurotoxicity of potassium cyanide could be overcome by concurrent melatonin administration. Indeed, both the seizures as well as the marked increase in neural lipid peroxidation were curtailed by the pharmacological administration of melatonin. A similar protective action of melatonin was seen in terms of the peroxidation of lipids when brain homogenate were incubated with cyanide.

The same group (Yamamoto and Yang, 1996c,d) examined melatonin's ability to resist oxidative damage to neural tissue induced by the sulfur-containing amino acid, L-cysteine. Like cyanide, L-cysteine administration causes seizures and the destruction of membrane lipids in the central nervous membrane. Since L-cysteine does not cross the blood–brain barrier, for the *in vivo* studies it was given intracerebroventricularly while melatonin was administered subcutaneously. These studies showed that the damaging effects of L-cysteine in the CNS were greatly attenuated by melatonin.

Lipopolysaccharide (LPS), a bacterial endotoxin which is highly toxic and which causes lipid peroxidation in many organs, can cause multiple organ failure (Yoshikawa *et al.*, 1994). LPS was used by Sewerynek *et al.* (1995a) to test melatonin's ability to protect against the oxidative breakdown of lipids. In the initial studies, which were carried out *in vitro*, the lipids in brain homogenates were protected from LPS-induced oxidative destruction by melatonin. When extended to the *in vivo* situation, melatonin similarly preserved the integrity of brain lipids otherwise destroyed by LPS (Sewerynek *et al.*, 1995b) and furthermore the indole prevented the drop in fluid intake and fever that was a consequence of bacterial endotoxin administration (Nava *et al.*, 1997). The dipsogenic effects of melatonin were believed, by the authors, to be due to a reduction in $NO^{\cdot}$ generation while it presumably reduced body temperature by preventing the excessive formation of prostaglandins and cytokines.

$H_2O_2$ readily generates the $^{\cdot}OH$ in tissue homogenates where transition metals are present. In neural homogenates melatonin resists the peroxidation of fatty acids induced by $H_2O_2$ (Sewerynek *et al.*, 1995c). Likewise, in the retina melatonin at pharmacological concentrations also prevents the peroxidation of lipids (Chen *et al.*, 1995). The studies on the retina are of special interests since melatonin is normally synthesized in this tissue. According to Longoni *et al.* (1997), while melatonin does inhibit lipid peroxidation in the retina, its precursor, that is, *N*-acetylserotonin (NAS), is even more effective in this regard and thus they consider endogenous NAS to be physiological relevant as an antioxidant in the neural retina.

There are obviously numerous neurotoxins against which melatonin, as a potentially protective agent, has not yet been tested. If an agent works via free radical mechanisms to inflict its damage, however, it is likely that melatonin will ameliorate the destruction. Among the studies reported to date, melatonin has not failed to resist the assault by free radicals, regardless of where and how they were generated.

## 5. FINAL COMMENTARY

Obviously, there have been a variety of procedures attempted to reduce cognitive decline and neuronal loss due to degenerative processes in the CNS (Fig. 10). The most favorable would be to prevent the damage before it occurs. The data summarized in this review illustrates how one molecule, that is, melatonin, experimentally at least is neuroprotective against a variety of degenerative processes in the brain and may be an agent that could prove beneficial in forestalling neurodegeneration by preventing the initial damage.

The bulk of the studies summarized herein clearly utilized pharmacological levels of melatonin to com-

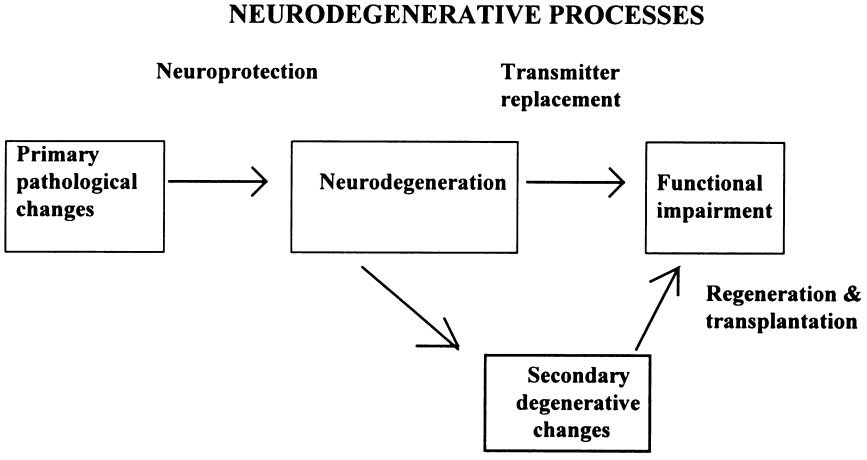


Fig. 10. Schematic representation of the processes of neurodegeneration that compromise the function of the CNS. Several means are potentially available to prevent or restore neural function. Ideally, agents that would provide neuroprotection would be best and probably least costly. Antioxidants, including melatonin, may have these neuroprotective functions.

bat the massive oxidative stress that was induced by a variety of procedures and agents. On the other hand one would not expect physiological levels of any antioxidant to prevent the free radical damage caused by the overwhelming insults which are summarized in this report. Indeed, the reason oxidative damage occurs under these circumstances is that endogenous, that is, physiological, defense mechanisms are incapable of resisting such enormous onslaughts of free radicals.

Melatonin's efficacy in combating free radical damage in the brain is certainly assisted by the ease with which it crosses the blood–brain barrier. Typically, melatonin was administered 20–30 min prior to the initiation of the free radical generating agent or process and it almost uniformly has proven effective in limiting the resulting neural damage. There are few if any, known antioxidants which, following a single administration, would be as powerful as melatonin as a protective agent against the very highly toxic agents and processes employed in the reports summarized herein.

This being the case, the possibility should be considered that melatonin does not function exclusively as a free radical scavenger and antioxidant but may have other actions which help cells and organisms to cope with metabolic disasters. While the investigations are still in their early phases, some studies have shown that melatonin influences gene expression beyond those related to antioxidative enzymes. For example, nuclear factor kappa B (NF$\kappa$B), a multifunctional transcription factor which is capable of influencing to a variety of genes, has been shown to be influenced by melatonin. Studies on melatonin's effects on gene regulation may help to uncover additional mechanisms whereby melatonin affords protection against free radicals.

The CNS obviously is highly vulnerable to free radical-induced molecular damage. This toxicity relates not only to the high production of these toxic agents but also due to its weaker than normal mechanisms to ward off such attacks. This probably accounts for the large number of neural diseases (Table 1) that are attributable to oxidative damage. Furthermore, free radical damage to critical brain structures probably plays a major role in neural deterioration with age. In view of this, melatonin should be considered for use in clinical trials which are designed to forestall the onset of free radical-related neurological diseases and neurobiological aging (Reiter, 1995c; Reiter *et al*., 1997c, 1998b). Investigations of this type seem especially relevant considering the great increase in longevity that humans are experiencing.

## REFERENCES


Acuña-Castroviejo, D., Coto-Montes, A., Monti, M. G., Ortiz, G. G. and Reiter, R. J. (1997) Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life Sci*. **60,** PL23–PL29.

Agarwal, M. L., Clay, M. E., Harvey, E. J., Evans, H. H., Antunez, A. R. and Oleineck, N. L. (1991) Phospholipase activation triggers apoptosis in photosensitized mouse lymphoma cells. *Cancer Res*. **51,** 5993–5996.

Aikens, J. and Dix, T. A. (1991) Perhydroxyl radical (HOO$^{\cdot}$) initiated lipid peroxidation: the role of fatty acid hydroperoxides. *J. Biol. Chem*. **266,** 15091–15098.

Aldhous, M., Franey, C., Wright, J. and Arendt, J. (1985) Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br. J. Clin. Pharmac*. **19,** 517–521.

Antolin, I., Rodriquez, C., Sainz, R. M., Mayo, J. C., Aria, H., Kotter, M., Rodriquez-Colungo, M. J., Tolivia, D. and Menendez-Pelaez, A. (1996) Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. *FASEB J*. **10,** 882–890.

Antolin, I., Obst, B., Burkhardt, S. and Hardeland, R. (1997) Antioxidant protection in a high-melatonin organism: the dinoflagellate *Gonyaulax polyedra* is rescued from lethal oxidative stress by strongly elevated but physiologically possible concentrations of melatonin. *J. Pineal Res*. **23,** 182–190.

Anton-Tay, F., Martinez, I., Tovar, R. and Benitz-King, G. (1998) Modulation of the subcellular distribution of calmodulin by melatonin in MDCK cells. *J. Pineal Res*. **24,** 35–42.

Asano, T., Matsui, T. and Takuwa, Y. (1991) Lipid peroxidation, protein kinase C and cerebral ischemia. *Crit. Rev. Neurosurg*. **1,** 361–379.

Bandy, B. and Davison, A. J. (1990) Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging. *Free Radical Biol. Med*. **18,** 523–539.

Barlow-Walden, L. R., Reiter, R. J., Abe, M., Pablos, M. I., Chen, L. D. and Peoggeler, B. (1995) Melatonin stimulates brain glutathione peroxidase activity. *Neurochem. Int*. **26,** 497–502.

Barreto, J. C., Smith, G. S., Strobel, N. H. P., McQuillin, P. A. and Miller, T. A. (1994) Terephthalic acid: a dosimeter for the protection of hydroxyl radicals *in vitro*. *Life Sci*. **56,** PL89–96.

Beal, M. F. (1995) Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann. Neurol*. **38,** 357–366.

Beckman, J. S. and Koppenol, W. H. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol*. **271,** CL427–437.

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. and Freeman, B. A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications of endothelial injury from nitric oxide and superoxide. *Proc. natl. Acad. Sci. U.S.A*. **87,** 1620–1624.

Beckman, J. S., Ischiropoulos, H., Zhu, L., van der Waal, M., Smith, C., Chen, J., Harrison, J., Martin, J. C. and Tsai, M. (1992) Kinetics of superoxide dismutase—an iron catalyzed nitration of phenolics by peroxynitrite. *Arch. Biochem. Biophys*. **298,** 438–455.

Beckman, K. B. and Ames, B. N. (1997) Oxidative decay of DNA. *J. Biol. Chem*. **272,** 19633–19636.

Ben-Shackar, D., Ashkenazi, R. and Youdim, M. B. H. (1986) Long term consequences of early iron deficiency on dopaminergic neurotransmission. *Int. J. Dev. Neurosci*. **4,** 81–88.

Bernheimer, H., Bukmayer, W., Hornykiewiez, O., Jellinger, K. and Stetelberger, F. (1973) Brain dopamine and the syndromes of Parkinson and Huntington. *J. Neurol. Sci*. **20,** 415–455.

Benzi, G. and Noretti, A. (1995) Are reactive oxygen species involved in Alzheimer's disease? *Neurobiol. Aging* **16,** 661–674.

Bertuglia, S., Marchiafava, P. L. and Colantuoni, A. (1996) Melatonin prevents ischemia reperfusion injury in the hamster check pauch. *Cardiovasc. Res*. **31,** 947–952.

Bettahi, I., Pozo, D., Osuna, C., Reiter, R. J., Acuña-Castroviejo, D. and Guerrero, J. M. (1996) Physiological concentrations of melatonin inhibit nitric oxide synthase activity in rat hypothalamus. *J. Pineal Res*. **20,** 205–210.

Bielski, B. H. J. (1991) Studies of hypervalent iron. *Free Radical Biol. Med*. **12,** 469–477.

Binkley, S. (1988) *The Pineal: Endocrine and Neuroendocrine Function*. Prentice Hall, Englewood Cliffs, NJ.

Bondy, S. C. (1997) Free-radical-mediated toxic injury to the nervous system. In: *Free Radical Toxicology*, pp. 221–248. Ed. K. B. Wallace. Taylor and Francis, Washington.

Braughler, J. M. and Hall, E. D. (1989) Central nervous system trauma and stroke. *Free Radical Biol. Med*. **6,** 289–301.

Bredt, D. S. and Synder, S. H. (1990) Isolation of nitric oxide synthase, a calmodulin requiring enzyme. *Proc. natl. Acad. Sci. U.S.A*. **87,** 682–685.

Burton, G. W., Joyce, A. and Ingold, K. V. (1983) Is vitamin E the only lipid-soluble, chain breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys*. **221,** 281–290.

Butterfield, D. A., Hersley, K., Harris, M., Mattson, M. P. and Carney, J. M. (1994) $\beta$-Amyloid peptide free radical fragments

initiate lipoperoxidation in a sequence specific fashion: implications to Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **200,** 710–715.

Cagnoli, C. M., Atabay, C., Kharlamova, E. and Manev, H. (1995) Melatonin protects neurons from singlet oxygen-induced apoptosis. *J. Pineal Res.* **18,** 222–226.

Cao, G., Alessio, H. M. and Cutler, R. G. (1993) Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **14,** 303–311.

Carneiro, R. C. G. and Reiter, R. J. (1998) Melatonin protects against lipid peroxidation induced by δ-aminolevulinic acid in rat cerebellum, cortex, and hippocampus. *Neuroscience* **82,** 293–299.

Cazevieille, C. and Osbourne, N. N. (1997) Retinal neurons containing kainate receptors are influenced by exogenous kainate and ischemia while neurons lacking these receptors are not—melatonin counteracts the effect of ischemia and kainate. *Brain Res.* **755,** 91–100.

Cazevieille, C., Sofa, R. and Osbourne, N. N. (1997) Melatonin protects primary cultures of rat cortical neurons from NMDA excitotoxicity and hypoxia/reoxygenation. *Brain Res.* **768,** 120–124.

Chan, P., DeLanny, L. E., Irwin, I., Langston, J. W. and DiMonte, D. (1991) Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse brain. *J. Neurochem.* **57,** 348–351.

Chan, T. Y. and Tang, P. L. (1996) Characterization of the antioxidant effects of melatonin and related indoleamines *in vitro*. *J. Pineal Res.* **20,** 187–191.

Chance, B., Sies, H. and Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59,** 527–605.

Cheesman, K. H. (1993) Tissue injury by free radicals. *Toxic. Indust. Health* **9,** 39–51.

Chen, L. D., Melchiorri, D., Sewerynek, E. and Reiter, R. J. (1995) Retinal Lipid peroxidation *in vitro* is inhibited by melatonin. *Neurosci. Res. Commun.* **17,** 151–158.

Cho, S., Joh, T. H., Baik, H. H., Dibinis, C. and Volpe, B. T. (1997) Melatonin administration protects CA1 hippocampal neurons after transient forebrain ischemia in rats. *Brain Res.* **755,** 335–338.

Choi, D. W. (1988) Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1,** 623–634.

Christensen, T., Bruhn, T., Balchen, T., Seitzberg, D. A., Jensen, B., Johnson, F. F. and Diemer, N. H. (1994) Detection of hydroxyl radicals in global cerebral ischemia by salicylate trapping and microdialysis. In: *Pharmacology of Cerebral Ischemia*, pp. 269–276. Eds. J. Krieglstein and H. Oberpichler-Schwenk. Medpharm, Stuttgart.

Cochrane, C. (1991) Mechanisms of oxidant injury of cells. *Mol. Aspects Med.* **12,** 137–147.

Cohen, G. (1988) Oxygen radicals and Parkinson,s disease. In: *Oxygen Radicals and Tissue Injury*, pp. 130–135. Ed. B. Halliwell. Federation of American Societies for Experimental Biology, Washington.

Cohen, G. and Heikkila, R. (1974) The generation of hydrogen peroxide, superoxide radical and hydroxyl radical by 6-hydroxydopamine, dialuric acid and related cytotoxic species. *J. Biol. Chem.* **250,** 2447–2452.

Costa, E. J. X., Lopes, R. H. and Lamy-Freund, M. T. (1995) Permeability of pure lipid bilayers to melatonin. *J. Pineal Res.* **19,** 123–126.

Costa, E. J. X., Shida, C. S., Biaggi, M. H., Ito, A. S. and Lamy-Freund, M. T. (1997) How melatonin interacts with lipid bilayers: a study by fluorescence and ESR spectroscopies. *FEBS Lett.* **416,** 103–106.

Coyle, J. T. and Puttfarcken, P. (1993) Oxidative stress, glutamate and neurodegenerative disorders. *Science* **262,** 689–695.

Crow, J. P., Beckman, J. S. and McCord, J. M. (1995) Sensitivity of the zinc-thiolate mioety of yeast alcohol dehydrogenase to hypochorite and peroxynitrite. *Biochemistry* **34,** 3544–3552.

Cuzzocrea, S., Zingarelli, B., Gilad, E., Hake, P., Salzman, A. L. and Szabo, C. (1997) Protective effect of melatonin in carregeenan-induced models of local inflammation: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. *J. Pineal Res.* **23,** 106–116.

Cuzzocrea, S., Zingarelli, B., Constantino, G. and Caputi, A. P. (1998) Protective effect of melatonin in a non-septic shock model induced by zymosan in the rat. *J. Pineal Res.* in press.

Daniels, W. M. V., van Rensburg, S. J., van Zyl, J. M. and Taljaard, J. J. F. (1998) Melatonin prevents β-amyloid-induced lipid peroxidation. *J. Pineal Res.* **24,** 78–82.

Dawson, T. M. and Snyder, S. H. (1994) Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J. Neurosci.* **14,** 1547–1559.

Dawson, T. M., Dawson, V. L. and Snyder, S. H. (1992) A novel neuronal messenger molecule in the brain: the free radical nitric oxide. *Ann. Neurol.* **32,** 297–311.

Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S. and Snyder, S. H. (1991) Nitric oxide mediates glutamate toxicity in primary cortical cultures. *Proc. natl. Acad. Sci. U.S.A.* **88,** 6371–6388.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P. and Marsden, C. D. (1989) Basal lipid peroxidation in substantia nigra increased in Parkinson's disease. *J. Neurochem.* **52,** 381–389.

Dipasquale, B., Marini, A. M. and Youle, R. (1991) Neuron apoptosis and DNA degradation induced by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridinium (MPP+). *Biochem. Biophys. Res. Commun.* **181,** 142–148.

Drijfhout, W. J., Homan, E. J., Brons, H. F., Oakley, N. R., Shingle, M., Grol, C. J. and Westernik, B. H. C. (1996) Exogenous melatonin entrains rhythms and reduces amplitude of endogenous melatonin: an *in vivo* microdialysis study. *J. Pineal Res.* **20,** 24–32.

Escames, G., Guerrero, J. M., Reiter, R. J., Garcia, J. J., Muñoz-Hoyos, A., Ortiz, G. G. and Oh, C. S. (1997) Melatonin and vitamin E limit nitric oxide-induced lipid peroxidation in rat brain homogenates. *Neurosci. Lett.* **230,** 147–150.

Esterbauer, H. (1985) Lipid peroxidation production: formation, chemical properties and biological activities. In: *Free Radicals and Liver Injury*, pp. 29–47. Eds. G. Poli, K. H. Cheeseman, M. V. Dianzani and T. F. Slater. IRL Press, Arlington, VA.

Evans, C., Scaiano, J. C. and Ingold, K. V. (1992) Absolute kinetics of hydrogen abstraction from α-tocopherol by several reactive species including an alkyl radical. *J. Am. Chem. Soc.* **14,** 4589–4593.

Fahn, S. and Cohen, G. (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann. Neurol.* **32,** 804–812.

Fearnley, J. M. and Lees, A. J. (1991) Aging and Parkinson's disease: substantia nigra regional selectivity. *Brain* **114,** 2283–2301.

Feldman, P. L., Griffith, O. W. and Stuehr, D. J. (1993) The surprising life of nitric oxide. *Chem. Eng. News* **12,** 26–38.

Finnochiaro, L. M. E. and Glikin, G. C. (1998) Intracellular melatonin distribution in cultured cell lines. *J. Pineal Res.* **24,** 22–34.

Floreani, J. M. and Lees, A. J. (1991) Aging and Parkinson's disease: substantia nigra regional selectivity. *Brain* **114,** 2283–2301.

Floyd, R. A. (1990) Role of oxygen free radicals in carcinogenesis and brain ischemia. *FASEB J.* **4,** 2587–2597.

Freeman, B. A. and Crapo, J. D. (1982) Biology of disease: free radicals and tissue injury. *Lab. Invest.* **47,** 412–426.

Fridovich, I. (1989) Superoxide dismutases: an adaptation to a paramagnetic gas. *J. Biol. Chem.* **264,** 19328–19333.

Garcia, J. J., Reiter, R. J., Guerrero, J. M., Escames, G., Yu, B. P., Oh, C. S. and Muñoz-Hoyos, A. (1997) Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett.* **408,** 297–300.

Garcia, J. J., Reiter, R. J., Ortiz, G. G., Oh, C. S., Tang, L., Yu, B. P. and Escames, G. (1998) Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. *J. Membr. Biol.* **162,** 59–65.

Ghosh, C., Dick, R. M. and Ali, S. F. (1993) Iron/ascorbate-induced lipid peroxidation changes membrane fluidity and muscarinic cholingeric receptor binding in rat frontal cortex. *Neurochem. Int.* **23,** 479–484.

Gilad, E., Cuzzocrea, S., Zingarelli, B., Salzman, A. L. and Szabo, C. (1997) Melatonin is a scavenger of peroxynitrite. *Life Sci.* **60,** PL169–PL174.

Giusti, P., Gusella, M., Lipartiti, M., Milani, D., Zhu, W., Vicini, S. and Manev, H. (1995) Melatonin protects primary cultures of cerebellar granule neurons from kainate but not from *N*-methyl-D-aspartate excitotxicity. *Exp. Neurol.* **131,** 39–46.

Giusti, P., Lipartiti, M., Franceschini, D., Shiavo, N., Floreani, M. and Manev, H. (1996) Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. *FASEB J.* **10,** 891–896.

Giusti, P., Lipartiti, M., Gusella, M., Floreani, M. and Manev, H. (1997) *In vitro* and *in vivo* protective effects of melatonin against glutamate oxidative stress and neurotoxicity. *Ann. N.Y. Acad. Sci.* **825,** 79–84.

Griffith, O. W. (1985) Glutathione and glutathione disulfide. In: *Methods of Enzymatic Analysis*, pp. 265–275. Ed. H. V. Bergmeyer. VCH Verlags-Gezellschaft, Weinheim.

Guerrero, J. M., Reiter, R. J., Ortiz, G. G., Pablos, M. I., Sewerynek, E. and Chuang, J. I. (1997) Melatonin prevents increases in neural nitric oxide and cycle GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (*Meriones unguiculatus*). *J. Pineal Res.* **23,** 24–31.

Hall, E. D., Andrus, P. K. and Yonkers, P. A. (1993) Brain hydroxyl radical generation in acute experimental head injury. *J. Neurochem.* **60,** 588–594.

Halliwell, B. (1978) Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. *FEBS Lett.* **92,** 321–326.

Halliwell, B. (1994) Free radicals and antioxidants: a personal view. *Nutr. Rev.* **52,** 253–265.

Halliwell, B. and Arouma, O. I. (1989) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* **281,** 9–19.

Halliwell, B. and Gutteridge, J. M. C. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219,** 1–14.

Halliwell, B. and Gutteridge, J. M. C. (1985) Oxygen radicals and the nervous system. *Trends Neurosci.* **8,** 22–26.

Halliwell, B. and Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine*, 2nd Edn. Clarendon, Oxford.

Halliwell, B. and Gutteridge, J. M. C. (1990) Role of free radicals and catalytic metal ions in human disease: a review. *Meth. Enzym.* **186,** 1–85.

Hard, G. C., Iatropoulos, M. J., Jordan, K., Radi, L., Kaltenberg, O. P., Imondi, A. R. and Williams, G. M. (1993) Major differences in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Grlicd (BR) rats. *Cancer Res.* **53,** 4534–4541.

Hardeland, R. (1997) New actions of melatonin and their relevance to biometeorology. *Int. J. Biometeorol.* **41,** 47–57.

Hardeland, R., Reiter, R. J., Poeggeler, B. and Tan, D. X. (1993) The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. *Neurosci. Biobehav. Rev.* **17,** 347–357.

Hardeland, R., Balzer, I., Poeggeler, B., Fuhrberg, B., Uria, H., Behrmann, R., Wolf, R., Meyer, T. J. and Reiter, R. J. (1995) On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photoxidation, and scavenging of free radicals. *J. Pineal Res.* **18,** 104–111.

Harman, D. (1980) Free radical theory of aging: origin of life, evolution, and aging. *Age* **3,** 100–102.

Harman, D. (1991) The aging process: major risk factor for disease and death. *Proc. natl. Acad. Sci. U.S.A.* **88,** 5360–5633.

Harman, D. (1992) Free radical theory of aging. *Mutat. Res.* **275,** 257–266.

Harman, D. (1995) Free radical theory of aging: Alzheimer's disease pathogenesis. *Age* **18,** 97–119.

Harris, M. E., Hensley, K., Butterfield, D. A., Leedle, R. A. and Carney, J. M. (1995) Direct evidence of oxidative injury produced by the Alzheimer's $\beta$-amyloid peptide (1–40) in cultured hippocampal neurons. *Exp. Neurol.* **131,** 193–202.

Hattori, A., Migitaka, H., Masayake, I., Itoh, M., Yamamoto, K., Ohtani-Kaneko, R., Hara, M., Suzuki, T. and Reiter, R. J. (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem. Mol. Biol. Int.* **35,** 627–634.

Henden, T., Stokkan, K. A., Reiter, R. J., Nonaka, K. O., Lerchl, A. and Jones, D. J. (1992) The age-associated reduction in pineal $\beta$-adrenergic receptor density is prevented by life-long food restriction. *Biol. Signals* **1,** 34–39.

Hensley, K., Hall, N., Subramanian, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, P., Wu, J. F., Carney, J. M., Lovell, M., Markesbery, W. R. and Butterfield, D. A. (1995) Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J. Neurochem.* **65,** 2146–2156.

Hensley, K., Pye, Q. N., Tabatabaie, T., Stewart, C. A. and Floyd, R. A. (1997) Reactive oxygen involvement in neurodegenerative pathways. In: *Neuroinflammation: Mechanisms and Management*, pp. 265–281. Ed. P. L. Wood. Humana Press, Totowa.

Holmes, S. W. and Sugden, D. (1976) The effect of melatonin on pinealectomy-induced hypertension in the rat. *Br. J. Pharmac.* **56,** 360P–361P.

Hurn, P. D., Kirsch, J. R., Helfaer, M. A. and Traystman, R. J. (1996) Brain damage and free radicals, In: *Tissue Perfusion and Organ Function: Ischemia/Reperfusion Injury*, pp. 71–84. Eds. T. Kamada, T. Shiga and R. S. McCuskey. Elsevier Science, New York.

Iacovitti, L., Stull, N. D. and Johnston, K. (1997) Melatonin rescues dopamine neurons from cell death in tissue culture models of oxidative stress. *Brain Res.* **768,** 317–326.

Ianas, O., Olivescu, R. and Badescu, I. (1991) Melatonin involvement in oxidative processes. *Rom. J. Endocrin.* **29,** 117–123.

Iguchi, H., Kato, M. and Ibayashi, H. (1982) Age-dependent reduction in serum melatonin concentrations in healthy individuals. *J. Clin. Endocrinol. Metab.* **55,** 27–29.

Imlay, J. A., Chin, S. M. and Linn, S. (1988) Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro*. *Science* **240,** 1302–1309.

Jain, A., Martensson, J., Stole, E., Auld, P. A. M. and Meister, A. (1991) Glutathione deficiency leads to mitochondrial damage in brain. *Proc. natl. Acad. Sci. U.S.A.* **88,** 1913–1917.

Jaskot, R. H., Charlet, E. G., Grose, E. C. and Grady, M. A. (1983) An automated analysis of glutathione peroxidase, S-transferase, and reductase activity in animal tissue. *J. Anal. Toxic.* **7,** 86–88.

Johnson, J. D., Conroy, W. G., Burris, K. D. and Isom, G. E. (1987) Peroxidation of brain lipids following cyanide intoxication in mice. *Toxicology* **46,** 21–28.

Kang, I., Lemair, H. G., Unterbeck, A., Salbaum, J. M., Masters, L. L., Grzeshik, K. H., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987) The precursor of Alzheimer's disease A4 protein resembles a cell–surface receptor. *Nature* **325,** 733–736.

Karppanen, H. (1974) Effect of propranolol on the blood pressure of normotensive and pinealectomized hypertensive rats. *Naunyn-Schmiedeberg's Arch. Pharmac.* **281,** 1–12.

Kawakara, M., Muramoto, K., Kobayaski, K. and Kuroda, Y. (1992) Functional and morphological changes in cultured neurons of rat cerebral cortex induced by long-term application of aluminum. *Biochem. Biophys. Res. Commun.* **189,** 1317–1322.

Kehrer, J. P. (1993) Free radicals, mediators of tissue injury and disease. *Crit. Rev. Toxic.* **23,** 21–48.

King, P. A., Anderson, V. E., Edwards, J. O., Gustafason, G., Plumb, R. C. and Sugus, J. W. (1992) A stable solid that generates hydroxyl radical upon dissolution in aqueous solution: reaction with proteins and nucleic acids. *J. Am. Chem. Soc.* **114,** 5430–5432.

Kinouchi, H., Epstein, C. J. and Mizae, T. (1991) Attenuation of focal cerebral ischemic injury in transgenic mice over expressing CuZn superoxide dismutase. *Proc. natl. Acad. Sci. U.S.A.* **88,** 11158–11162.

Klaunig, J. E., Xu, Y., Bachowski, S. and Jiang, J. (1997) Free-radical oxygen-induced changes in chemical carcinogenesis. In: *Free Radical Toxicology*, pp. 375–400. Ed. K. B. Wallace. Taylor and Frances, Washington.

Koh, J., Yang, L. L. and Cotman, C. W. (1990) $\beta$-Amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Res.* **533,** 315–320.

Köhler, C. (1984) Neuronal degeneration after intracerebral injections of excitotoxins. A histological analysis of kainic acid, ibotenic acid and quinolinic acid lesions in the brain. In: *Excitotoxins*, pp. 99–111. Eds. K. Fuke, P. Roberts and R. Schwarz. Plenum, New York.

Kotler, M., Rodriquez, C., Sainz, R. M., Antolin, I. and Menendez-Pelaez, A. (1998) Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J. Pineal Res.* **24,** 83–89.

Krause, D. N., Barrios, V. E. and Duckles, S. P. (1995) Melatonin receptors mediate potentiation of contractile responses to adrenergic nerve stimulation in rat caudal artery. *Eur. J. Pharmac.* **276,** 207–213.

Krieglstein, J. and Oberpichler-Schwenk, H. (1994) *Pharmacology of Cerebral Ischemia*. Medpharm, Stuttgart.

Lai, M., Griffiths, H., Pall, H., Williams, A. and Lunec, J. (1993) An investigation into the role of reactive oxygen species in mechanisms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity using neuronal cell lines. *Biochem. Pharmac.* **45,** 927–933.

Lees, A. J. (1992) When did Ray Kennedy's Parkinson's disease being? *Movement Discord.* **7,** 110–116.

Leigh, G. H. (1990) *Nomenclature of Inorganic Chemistry*. Blackwell Scientific, Oxford.

Levine, R. L. and Stadtman, E. R. (1992) Oxidation of proteins during aging. *Generations* **14,** 39–42.

Lezoualc'h, F., Skutella, T., Widmann, M. and Behl, C. (1996) Melatonin prevents oxidative stress-induced cell death in hippocampal cells. *NeuroReport* **7,** 2071–2077.

Lezoualc'h, F., Sparapani, M. and Behl, C. (1998) *N*-Acetyl-serotonin (normelatonin) and melatonin protect neurons against

oxidative challenges and suppress the activity of the transcription factor NF-κB. *J. Pineal Res.* **24,** 168–178.

Li, X. J., Zhang, L. M., Gu, J., Zhang, A. Z. and Sun, F. Y. (1997) Melatonin decreases production of hydroxyl radical during ischemia-reperfusion. *Acta Pharmac. Sinica* **18,** 394–396.

Liebler, D. C. (1993) Peroxyl radical trapping reactions of α-tocopherol in biomimetic systems. In: *Vitamin E in Health and Disease*, pp. 85–95. Eds. L. Packer and J. Fuchs. Dekker, New York.

Limson, J., Nyokong, T. and Daya, S. (1998) The interaction of melatonin and its precursors with aluminum, cadmium, copper, iron, lead, and zinc: an absorptive voltammetric study. *J. Pineal Res.* **24,** 15–21.

Lipton, S. A., Choi, Y. B., Pan, Z. H., Lei, S. Z. and Chen, H. V. (1993) A redox-based mechanism in the neuroprotective and neurodestructive effect of nitric oxide and related nitroso-compounds. *Nature* **364,** 626–632.

Longoni, B., Pryor, W. A. and Marchiafava, P. (1997) Inhibition of lipid peroxidation by *N*-acetyserotonin and its role in retinal physiology. *Biochem. Biophys. Res. Commun.* **233,** 778–780.

Löschmann, P. A., Lange, K. W., Wachtel, H. and Turski, L. (1994) MPTP-induced degeneration: interference with glutaminergic activity. *J. Neural Transm.* **43**(Suppl), 133–143.

Mainous, M. R., Ertel, W., Chaudry, I. H. and Deitch, E. A. (1995) The gut: a cytokine-generating organ in system inflammation. *Shock* **4,** 193–199.

Malinski, T., Bailey, F., Zhang, Z. G. and Chopp, M. (1993) Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* **13,** 355–359.

Manchester, L. C., Poeggeler, B., Alvares, F. L., Ogden, G. B. and Reiter, R. J. (1995) Melatonin immunoreactivity in the photosynthetic prokaryote, *Rhodospirillum rubrum*: implications for an ancient antioxidant system. *Cell. Mol. Biol. Res.* **41,** 391–395.

Manelli, A. M. and Puttfarcken, P. S. (1995) β-Amyloid-induced toxicity in rat hippocampal cells: *in vitro* evidence for the involvement of free radicals. *Brain Res. Bull.* **38,** 569–576.

Manev, H. Uz T., Kharlamov, A., Cagnoli, C. M., Franseschini, D. and Giusti, P. (1996a) *In vivo* protection against kainate-induced apoptosis by the pineal hormone melatonin: effect of exogenous melatonin and circadian rhythm. *Restr. Neurol. Neurosci.* **9,** 251–256.

Manev, H., Uz, T., Kharlamov, A. and Joo, J. Y. (1996b) Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. *FASEB J.* **10,** 1546–1551.

Markesbery, W. R. (1997) Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **23,** 134–137.

Marshall, K. A., Reiter, R. J., Poeggeler, B., Aruoma, O. I. and Halliwell, B. (1996) Evaluation of the antioxidant activity of melatonin *in vitro*. *Free Radical Biol. Med.* **21,** 307–315.

Mattson, M. P., Mark, R. J., Furukawa, K. and Bruce, A. J. (1997) Disruption of brain cell ion homeostasis in Alzheimer's disease by oxy radicals, and signaling pathways that protect therefrom. *Chem. Res. Toxic.* **10,** 507–517.

Matuszak, Z., Reszka, K. J. and Chignell, C. F. (1997) Reaction of melatonin and related indoles with hydroxyl radicals: ESR and spin trapping investigations. *Free Radical Biol. Med.* **23,** 367–372.

Mayo, J. C., Sainz, R. M., Uria, H., Antolin, I., Esteban, M. M. and Rodriquez, C. (1998a) Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. *J. Pineal Res.* **24,** 179–192.

Mayo, J. C., Sainz, R. M., Uria, H., Antolin, I., Esteban, M. M. and Rodriquez, C. (1998b) Inhibition of cell proliferation: a mechanism likely to mediate prevention of neuronal cell death by melatonin. *J. Pineal Res.* **24,** 179–192.

Meister, A. (1988) Glutathione metabolism and its selective modification. *J. Biol. Chem.* **263,** 17205–17208.

Melchiorri, D., Reiter, R. J., Sewerynek, E., Chen, L. D. and Nistico, G. (1995) Melatonin reduces kainate-induced lipid peroxidation in homogenates of different brain regions. *FASEB J.* **9,** 1205–1210.

Melchiorri, D., Reiter, R. J., Chen, L. D., Sewerynek, E. and Nistico, G. (1996) Melatonin affords protection against kainate-induced *in vitro* lipid peroxidation in brain. *Eur. J. Pharmac.* **305,** 239–242.

Menendez-Pelaez, A. and Reiter, R. J. (1993) Distribution of melatonin in mammalian tissues: relative importance of nuclear verses cytosolic localization. *J. Pineal Res.* **15,** 59–69.

Menendez-Pelaez, A., Poeggeler, B., Reiter, R. J., Barlow-Walden, L. R., Pablos, M. I. and Tan, D. X. (1993) Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. *J. Cell. Biochem.* **53,** 572–582.

Mésenge, C., Margaill, I. Verrecchia, C., Allix, M., Boulu, R. G. and Plotkine, M. (1998) Protective effect of melatonin in a model of traumatic brain injury in mice. *J. Pineal Res.* in press.

Michel, P. P. and Hefty, F. (1990) Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. *J. Neurosci. Res.* **26,** 428–435.

Miller, J. W., Selhub, J. and Joseph, J. A. (1996) Oxidative damage caused by free radicals produced during catecholamine autoxidation: protective effects of *O*-methylation and melatonin. *Free Radical Biol. Med.* **21,** 241–249.

Moncada, S., Palmer, R. M. J. and Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmac. Rev.* **43,** 109–143.

Montilla, P., Tunez, I., Muñoz, M. C., Soria, J. V. and Lopez, A. (1997) Antioxidative effect of melatonin in rat brain oxidative stress induced by adriamycin. *J. Physiol. Biochem.* **53,** 301–306.

Moore, M. R. (1993) Biochemistry of porphyria. *Int. J. Biochem.* **25,** 1353–1368.

Moreno, J. J. and Pryor, W. A. (1992) Inactivation of α-1-proteinase inhibitor by peroxynitrite. *Chem. Res. Toxic.* **5,** 425–431.

Murch, S. J., Simmons, C. B. and Saxena, P. K. (1997) Melatonin in feverfew and other medicinal plants. *Lancet* **350,** 1598–1599.

Nakanishi, S. (1992) Molecular diversity of glutamate receptors and implications for brain function. *Science* **258,** 597–603.

Nakazono, K., Watanabe, N., Matsuno, K., Sasaki, J., Sato, T. and Inoul, M. (1991) Does superoxide underlay the pathogenesis of hypertension? *Proc. natl. Acad. Sci. U.S.A.* **88,** 10045–10048.

Nava, F., Calapai, G., Facciola, G., Cuzzocrea, S., Giuliani, G., DeSarro, A. and Caputi, A. P. (1997) Melatonin effects on inhibition of thrist and fever induced by lipopolysaccharide in rat. *Eur. J. Pharmac.* **331,** 267–274.

Nohl, H. and Hegner, D. (1978) Do mitochondria produce oxygen free radicals *in vivo*? *Eur. J. Biochem.* **82,** 563–567.

Novelli, A., Reilly, J. A., Lysko, P. G. and Henneberry, R. C. (1988) Glutamate becomes neurotoxic via the *N*-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res.* **451,** 205–212.

O'Banion, M. K., Coleman, P. D. and Callahan, L. M. (1994) Regional neuronal loss in aging and Alzheimer's disease: a brief review. *Sem. Neurosci.* **6,** 307–314.

Ohtsuki, T., Matsumoto, M., Hayachi, Y., Yamamoto, K., Kitogawa, K., Ogawa, S., Yamamoto, S. and Kamada, T. (1995) Reperfusion induces 5-lipoxygenase translocation and leukotiene C4 production in ischemic brain. *Am. J. Physiol.* **268,** H1249–H1257.

Olanow, C. W. (1990) Oxidation reactions in Parkinson's disease. *Neurology* **40**(Suppl. 3), 32–37.

Orrenius, S., McAnkey, D. J., Bellomo, G. and Nicotera, P. (1989) Role of $Ca^{2+}$ in toxic cell killing. *Trends Pharmac. Sci.* **10,** 281–285.

Pablos, M. I., Agapito, M. T., Gutierrez, R., Recio, J. M., Reiter, R. J., Barlow-Walden, L. R., Acuña-Castroviejo, D. and Menendez-Pelaez, A. (1995a) Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J. Pineal Res.* **19,** 111–115.

Pablos, M. I., Chuang, J. I., Reiter, R. J., Ortiz, G. G., Daniels, W. M. U., Sewerynek, E., Melchiorri, D. and Poeggeler, B. (1995b) Time course of melatonin-induced increase in glutathione peroxidase activity in chick tissues. *Biol. Signals* **4,** 325–330.

Pablos, M. I., Guerrero, J. M., Ortiz, G. G., Agapito, M. T. and Reiter, R. J. (1997a) Both melatonin and a putative nuclear melatonin receptor agonist CGP 52608 stimulate glutathione peroxidase and glutathione reductase activities in mouse brain *in vivo*. *Neuroendocrin. Lett.* **18,** 49–58.

Pablos, M. I., Reiter, R. J., Chuang, J. I., Ortiz, G. G., Guerrero, J. M., Sewerynek, E., Agapito, M. T., Melchiorri, D., Lawrence, R. and Deneke, S. M. (1997b) Acutely administered melatonin reduces oxidative damage in lung and brain induced by hyperbaric oxygen. *J. Appl. Physiol.* **83,** 354–558.

Pablos, M. I., Reiter, R. J., Ortiz, G. G., Guerrero, J. M., Agapito, M. T., Chuang, J. I. and Sewerynek, E. (1998) Rhythms of glutathione peroxidase and glutathione reductase in brain of chicks and their inhibition by light. *Neurochem. Int.* **32,** 69–75.

Packer, L. (1994) Vitamin E is nature's master antioxidant. *Sci. Am. (Sci. Med.)* **Mar/Apr,** 54–63.

Pähkla, R., Zilmer, M., Kullisaar, T. and Rägo, L. (1998) Comparison of the antioxidant activity of melatonin and pinoline *in vitro*. *J. Pineal Res.* **24,** 96–101.

Pappolla, M. A., Sas, M., Omar, R. A., Bick, R. J., Hickson-Bick, D. L. M., Reiter, R. J., Efthimiopoulos, S. and Robakis, N. K. (1997a) Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide. *J. Neurosci.* **17,** 1683–1690.

Pappolla, M. A., Sas, M., Bick, R. J., Omar, R. A., Hickson-Bick, D. L. M., Reiter, R. J., Efthimiopoulous, S., Sambamurti, K. and Rubakis, N. K. (1997b) Oxidative damage and cell death induced by an amyloid peptide fragment is completely prevented by melatonin. In: *Alzheimer's Disease: Biology, Diagnosis and Therapeutics*, pp. 741–749. Eds. K. Iqbal, B. Winblad, T. Nishimura, M. Takeda and H. M. Wisniewski. Wiley, New York.

Pappolla, M. A., Soto, C., Bozner, P., Frangione, B. and Ghiso, J. (1997c) Disruption of $\beta$-fibrillogenesis by the pineal hormone melatonin. *Abstr. Soc. Neurosci.*, Abstract No. 731.1.

Pappolla, M. A., Bozner, P., Soto, C., Shao, H., Robakis, N. K., Zagorski, M., Frangione, B. and Ghiso, J. (1998) Inhibition of Alzheimer's $\beta$-fibrillogenesis by melatonin. *J. Biol. Chem.* in press.

Perry, T. L. and Young, V. W. (1986) Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. *Neurosci. Lett.* **67,** 269–274.

Pieri, C., Marra, M., Moroni, F., Recchioni, R. and Marcheselli, F. (1994) Melatonin: a peroxyl radical scavenger more effective than vitamin E. *Life Sci.* **55,** PL271–PL276.

Pieri, C., Moroni, F., Marra, M., Marcheselli, F. and Recchioni, R. (1995) Melatonin is an efficient antioxidant. *Arch. Gerontol. Geriatrics* **20,** 159–165.

Pierrefiche, G. and Laborit, H. (1995) Oxygen radicals, melatonin and aging. *Exp. Gerontol.* **30,** 213–227.

Poeggeler, B., Balzer, I., Hardeland, R. and Lerchl, A. (1991) Pineal hormone melatonin oscillates also in the dinoflagellate, *Gonyaulax polyedra*. *Naturwissenschaften* **78,** 268–269.

Poeggeler, B., Reiter, R. J., Tan, D. X., Chen, L. D. and Manchester, L. C. (1993) Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis. *J. Pineal Res.* **14,** 151–168.

Poeggeler, B., Saarela, S., Reiter, R. J., Tan, D. X., Chen, L. D., Manchester, L. C. and Barlow-Walden, L. R. (1994) Melatonin—a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole assessed *in vitro*. *Ann. N.Y. Acad. Sci.* **738,** 419–420.

Poeggeler, B., Reiter, R. J., Hardeland, R., Sewerynek, E., Melchiorri, D. and Barlow-Walden, L. R. (1995) Melatonin, a mediator of electron transfer and repair reactions, acts synergistically with the chain-breaking antioxidants ascorbate, trolox and glutathione. *Neuroendocrin. Lett.* **17,** 87–92.

Poeggeler, B., Reiter, R. J., Hardeland, R., Tan, D. X. and Barlow-Walden, L. R. (1996) Melatonin and structurally-related, endogenous indoles act as potent electron donors and radical scavengers *in vitro*. *Redox Report* **2,** 179–184.

Pozo, D., Reiter, R. J., Calvo, J. R. and Guerrero, J. M. (1994) Physiological concentrations of melatonin inhibit nitric oxide synthase in rat cerebellum. *Life Sci.* **55,** PL455–PL460.

Pozo, D., Reiter, R. J., Calvo, J. R. and Guerrero, J. M. (1997) Inhibition of cerebellar nitric oxide synthase and cyclic GMP production by melatonin via complex formation with calmodulin. *J. Cell. Biochem.* **65,** 430–442.

Princ, F. G., Juknat, A. A., Maxit, A. G., Cardalda, C. and Batlle, A. (1997) Melatonin's antioxidant protection against $\delta$-aminolevulinic acid-induced oxidative damage in the rat cerebellum. *J. Pineal Res.* **23,** 40–46.

Princ, F. G., Maxit, A. G., Cardala, C., Batlle, A. and Juknat, A. A. (1998) *In vivo* protection by melatonin against $\delta$-aminolevulinic acid-induced oxidative damage and its antioxidant effect on the activity of haem enzymes. *J. Pineal Res.* **24,** 1–8.

Pryor, W. and Squadrito, G. (1995) The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* **268,** L699–L722.

Puy, H., Deybach, J. C., Baudry, J., Touitou, Y. and Nordmann, Y. (1993) Decreased nocturnal plasma melatonin levels in patients with recurrent acute intermittent porphyria attacks. *Life Sci.* **53,** 621–627.

Puy, H., Deybach, J. C., Bogdan, A., Callebert, J., Baumgartner, M., Voisin, P., Nordmann, Y. and Touitou, Y. (1996) Increased $\delta$-aminolevulinic acid and decreased melatonin production. *J. Clin. Invest.* **97,** 104–110.

Radi, R., Beckman, J. S., Bush, K. M. and Freeman, B. A. (1991a) Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* **266,** 4244–4250.

Radi, R., Beckman, J. S., Bush, K. M. and Freeman, B. A. (1991b) Peroxynitrite induced membrane lipid peroxidation. The cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* **288,** 481–487.

Reiter, R. J. (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocrine Rev.* **12,** 151–180.

Reiter, R. J. (1992) The aging pineal gland and its physiological consequences. *Bio Essays* **14,** 169–175.

Reiter, R. J. (1995a) Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J.* **9,** 526–533.

Reiter, R. J. (1995b) Functional pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front. Neuroendocrinol.* **16,** 385–415.

Reiter, R. J. (1995c) Oxygen radical detoxification processes during aging: the functional importance of melatonin. *Aging Exp. Clin. Res.* **7,** 340–351.

Reiter, R. J. (1997a) Antioxidant actions of melatonin. *Adv. Pharmac.* **38,** 103–117.

Reiter, R. J. (1997b) Aging and oxygen toxicity: relation to changes in melatonin. *Age*.

Reiter, R. J., Richardson, B. A., Johnson, L. Y., Ferguson, B. N. and Dinh, D. T. (1980) Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science* **210,** 1372–1373.

Reiter, R. J., Craft, C. M., Johnson, J. E., Jr, King, T. S., Richardson, B. A., Vaughan, G. M. and Vaughan, M. K. (1981) Age-associated reduction in nocturnal pineal melatonin levels in female rats. *Endocrinology* **109,** 1295–1297.

Reiter, R. J., Tan, D. X., Poeggeler, B., Menendez-Peloez, A., Chen, L. D. and Saarela, S. (1994) Melatonin as a free radical scavenger: implications for aging and age-related diseases. *Ann. N.Y. Acad. Sci.* **719,** 1–12.

Reiter, R. J., Melchiorri, D., Sewerynek, E., Poeggeler, B., Barlow-Walden, L. R., Chuang, J. I., Ortiz, G. G. and Acuña-Castroviejo, D. (1995) A review of the evidence supporting melatonin's role as an antioxidant. *J. Pineal Res.* **18,** 1–11.

Reiter, R. J., Pablos, M. I., Agapito, T. T. and Guerrero, J. M. (1996a) Melatonin in the context of the free radical theory of aging. *Ann. N.Y. Acad. Sci.* **786,** 362–378.

Reiter, R. J., Oh, C. S. and Fujimori, O. (1996b) Melatonin: its intracellular and genomic actions. *Trends Endocrin. Metab.* **7,** 22–27.

Reiter, R. J., Tang, L., Garcia, J. J. and Muñoz-Hoyos, A. (1997a) Pharmacological actions of melatonin in oxygen radical pathophysiology. *Life Sci.* **60,** 2255–2271.

Reiter, R. J., Carneiro, R. C. and Oh, C. S. (1997b) Melatonin in relation to cellular antioxidative defense mechanisms. *Horm. Metab. Res.* **29,** 363–372.

Reiter, R. J., Guerrero, J. M., Escames, G., Pappolla, M. A. and Acuña-Castroviejo, D. (1997c) Prophylactic actions of melatonin in oxidative neurotoxicity. *Ann. N.Y. Acad. Sci.* **825,** 70–78.

Reiter, R. J., Pie, J. and Garcia, J. J. (1998a) Oxidative toxicity in models of neurodegeneration: responses to melatonin. *Restor. Neurol. Neurosci.* in press.

Reiter, R. J., Guerrero, J. M., Garcia, J. J. and Acuña-Castroviejo, D. (1998b) Reactive oxygen intermediates, molecular damage and aging: relation to melatonin. *Ann N. Y. Acad. Sci.* in press.

Richardson, J. S. and Zhou, Y. (1996) Oxidative stress in the production and expression of neurotoxic $\beta$-amyloid. *Restr. Neurol. Neurosci.* **9,** 207–211.

Robakis, N. K. and Pappolla, M. A. (1994) Oxygen-free radicals and amyloidosis in Alzheimer's disease: is there a connection? *Neurobiol. Aging* **15,** 457–459.

Sack, R. L., Lewy, A. J., Erb, D. L., Vollmer, W. M. and Singer, C. M. (1986) Human melatonin production decreases with age. *J. Pineal Res.* **3,** 379–388.

Sadrzadeh, S. M., Anderson, D. K., Panter, S. S., Hallaway, P. E. and Eaton, J. W. (1987) Hemoglobin potentiates central nervous system damage. *J. Clin. Invest.* **79,** 662–664.

Saran, M., Michel, C. and Bors, W. (1990) Reaction of NO with $O_2^{-\cdot}$: implications for the action of endothelium-releasing factor. *Free Radical Res. Commun.* **10,** 221–226.

Savolainen, H. (1978) Superoxidase dismutase and glutathione peroxidase activities in rat brain. *Res. Commun. Chem. Pathol. Pharmac.* **21,** 173–176.

Scaiano, J. C. (1995) Exploratory laser flash photolysis study of free radical reactions and magnetic field effects in melatonin chemistry. *J. Pineal Res.* **19,** 189–195.

Schulz, J. B., Henshaw, D. R., Siwek, D., Jenkins, B. G., Ferrante, R. J., Cippoloni, P. B., Kowall, N. W., Rosen, B. R. and Beal, M. F. (1995) Involvement of free radicals in excitotoxicity *in vivo*. *J. Neurochem.* **64,** 2239–2247.

Selkoe, D. J. (1994) Amyloid beta-protein and the genetics of Alzheimer's disease. *A. Rev. Neurosci.* **17,** 489–517.

Sevanian, A. and McLeod, L. (1997) Formation and biological reactivity of lipid peroxidation products. In: *Free Radical Toxicology*, pp. 47–70. Ed. K. B. Wallace. Taylor and Francis, Washington.

Sewerynek, E., Melchiorri, D., Chen, L. D. and Reiter, R. J. (1995a) Melatonin reduces both basal and bacterial lipopolysaccharide-induced lipid peroxidation *in vitro*. *Free Radical Biol. Med.* **19,** 903–909.

Sewerynek, E., Abe, M., Reiter, R. J., Barlow-Walden, L. R., Chen, L. D., McCabe, T. J., Roman, L. J. and Diaz-Lopez, B. (1995b) Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals. *J. Cell. Biochem.* **58,** 436–444.

Sewerynek, E., Melchiorri, D., Ortiz, G. G., Poeggeler, B. and Reiter, R. J. (1995c) Melatonin reduces $H_2O_2$-induced lipid peroxidation in homogenates of different rat brain regions. *J. Pineal Res.* **19,** 51–56.

Shaikh, A. Y., Xu, J., Wu, Y., He, L. and Hsu, C. Y. (1997) Melatonin protects bovine cerebral endothelial cells from hyperoxia-induced DNA damage and death. *Neurosci. Lett.* **229,** 193–197.

Shida, C. S., Castrucci, A. M. L. and Larmy-Freund, M. T. (1994) High melatonin solubility in aqueous medium. *J. Pineal Res.* **16,** 198–201.

Sies, H. (1993) Strategies of antioxidative defense. *Eur. J. Biochem.* **215,** 213–219.

Sies, H. and Stahl, W. (1995) Vitamins E and C, $\beta$-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr.* **62**(Suppl.), 1315S–1321S.

Sohal, R. S. (1997) Mitochondria generate superoxide anion radicals and hydrogen peroxide. *FASEB J.* **11,** 1309–1315.

Song, W. and Lahiri, D. K. (1997) Melatonin alters the metabolism of the $\beta$-amyloid precursor protein in the neuroendocrine cell line PC12. *J. Mol. Neurosci.* **9,** 75–92.

Stadtman, E. R. (1992) Protein oxidation and aging. *Science* **257,** 1220–1224.

Stasica, P., Ulanski, P. and Rosiak, J. M. (1998) Melatonin as a hydroxyl radical scavenger. *J. Pineal Res.* in press.

Stokkan, K. A., Reiter, J. R., Nonaka, K. D., Lerchl, A. and Yu, B. P. (1991) Food restriction retards aging of the pineal gland. *Brain Res.* **545,** 66–72.

Susa, N., Ueno, S., Furukawa, Y., Ueda, J. and Sugiyama, M. (1997) Potent protective effect of melatonin on chromium (VI)-induced DNA single-strand breaks, cytotoxicity, and lipid peroxidation in primary cultures of rat hepotocytes. *Toxic. Appl. Pharmac.* **144,** 377–384.

Szabo, C. (1996) The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia-reperfusion injury. *Shock* **6,** 79–88.

Tan, D. X., Chen, L. D., Poeggeler, B., Manchester, L. C. and Reiter, R. J. (1993a) Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrine J.* **1,** 57–60.

Tan, D. X., Poeggeler, B., Reiter, R. J., Chen, L. D., Chen, S., Manchester, L. C. and Barlow-Walden, L. R. (1993b) The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole *in vivo*. *Cancer Lett.* **70,** 65–71.

Tan, D. X., Reiter, R. J., Chen, L. D., Poeggeler, B., Manchester, L. C. and Barlow-Walden, L. R. (1994) Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. *Carcinogenesis* **15,** 215–218.

Tang, P. L., Chan, T. Y. and Zian, Z. M. (1996) Melatonin as an antioxidant: an assessment of its properties *in vitro*. In: *Melatonin: A Universal Photoperiodic Signal with Diverse Actions*, pp. 167–173. Eds. P. L. Tang, S. F. Pang and R. J. Reiter. Karger, Basel.

Tilden, A. R., Becker, M. A., Amma, L. L., Arceniega, J. and McGaw, A. K. (1997) Melatonin production in a aerobic photosynthetic bacterium: an evolutionary early association with darkness. *J. Pineal Res.* **22,** 102–106.

Torbati, D., Church, D. F., Keller, J. M. and Pryor, W. A. (1992) Free radical generation in the brain precedes hyperbaric oxygen-induced comulsions. *Free Radical Biol. Med.* **13,** 101–106.

Touati, D. (1989) The molecular genetics of superoxide dismutase in *E. coli*. An approach to understanding the biological role and regulation of SODs in relation to other elements of the defense system against oxygen toxicity. *Free Radical Biol. Med.* **8,** 1–9.

Touitou, V., Fevre, M., Langugvey, M., Carayon, A., Boydan, A. and Reinhert, A. (1981) Age and mental health related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle stimulating hormone. *J. Endocrin.* **9,** 467–475.

Urano, S. and Matsuo, M. (1989) Membrane-stabilizing effect of vitamin E. *Ann. N.Y. Acad. Sci.* **570,** 524–526.

Uz, T., Giusti, P., Franceschini, D., Kharlamov, A. and Manev, H. (1996) Protective effect of melatonin against hippocampal DNA damage induced by intraperitoneal administration of kainate to rats. *Neuroscience* **73,** 631–636.

Uz, T., Longone, P. and Manev, H. (1997) Increased hippocampal 5-lipoxygenase mRNA content in melatonin-deficient, pinealectomized rats. *J. Neurochem.* **69,** 2220–2223.

Vatassery, G. T. (1997) Vitamin E. Neurochemical aspects and relevance to nervous system disorders. In: *Metals and Oxidative Damage in Neurological Disorders*, pp. 175–188. Ed. P. M. Conner. Plenum, New York.

Victor, T., van der Merwe, N., Benadi, A. J. S., LaCock, C. and Lochner, A. (1985) Mitochondrial phospholipid composition and microviscosity in myocardial ischemia. *Biochem. Biophys. Acta* **834,** 215–223.

Vivien-Roels, B. and Pevet, P. (1993) Melatonin presence and formation in invertebrates. *Experientia* **49,** 642–647.

Wallace, D. C. (1992) Mitochondrial genetics: a paradigm for aging and degenerative diseases. *Science* **256,** 628–632.

Watanabe, H., Kobayashi, A., Yamamoto, T., Suzuki, S., Hayashi, H. and Yamazaki, N. (1990) Alterations of human erythrocyte membrane fluidity by oxygen-derived free radicals and calcium. *Free Radical Biol. Med.* **9,** 507–514.

Wei, J., Huang, N. G. and Quest, M. J. (1997) Hydroxyl radical formation in hyperglycemic rats during middle cerebral artery occulsion/reperfusion. *Free Radical Biol. Med.* **23,** 986–995.

Yamamoto, H. A. and Yang, H. W. (1996a) Preventive effect of melatonin against cyanide-induced seizures and lipid peroxidation in mice. *Neurosci. Lett.* **207,** 89–92.

Yamamoto, H. A. and Yang, H. W. (1996b) Antagonistic effect of melatonin against cyanide-induced seizures and acute lethality in mice. *Toxic. Lett.* **87,** 19–24.

Yamamoto, H. A. and Yang, H. W. (1996c) Effect of melatonin, piperonyl butoxide, or cobalt chloride on L-cysteine-induced lipid peroxidation in homogenate from whole brain of mice. *Toxic. Lett.* **89,** 51–56.

Yamamoto, H. A. and Yang, H. W. (1996d) Melatonin attenuates L-cysteine-induced seizures and lipid peroxidation in the brain of mice. *J. Pineal Res.* **21,** 108–113.

Yamasaki, I. and Piette, L. H. (1991) EPR spin trapping study on the oxidizing species formed in the reaction of the ferrous ion with hydrogen peroxide. *J. Am. Chem. Soc.* **113,** 7588–7593.

Yeleswaram, K., McLaughlin, L. G., Knipe, J. O. and Schabdoch, D. (1997) Pharmacokinetics and oral bioavailability of exogenous melatonin in preclinical animal models and clinical implications. *J. Pineal Res.* **22,** 45–51.

Yoshikawa, K. (1993a) Neurotoxicity of $\beta$-amyloid. *Nature* **361,** 122–123.

Yoshikawa, T. (1993b) Free radicals and their scavengers in Parkinson's disease. *Eur. Neurol.* **33**(Suppl 1), 60–68.

Yoshikawa, Y., Takano, H., Takahashi, S., Ichikawa, H. and Kondo, M. (1994) Changes in tissue antioxidant enzyme activities and lipid peroxides in endotoxin-induced multiple organ failure. *Circul. Shock* **42,** 53–58.

Youdim, M. B. H., Ben-Shackar, D., Eshel, G., Finberg, J. P. M. and Riederer, P. (1993) The neurotoxicity of iron and nitric oxide. *Adv. Neurol.* **60,** 259–266.

Zhang, J., Dawson, V. L., Dawson, T. M. and Snyder, S. H. (1994) Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* **263,** 687–689.

Zhang, Y., Marcillar, O., Giulivi, C., Eymster, L. and Davis, K. J. (1990) The oxidative inactivation of mitochondrial electron transport chain components of ATPase. *J. Biol. Chem.* **265,** 16330–16336.

Zigmond, M. J. and Sticker, E. M. (1989) Animal models of Parkinsonism using selective neurotoxins: clinical and basic implications. *Int. Rev. Neurbiol.* **31,** 1–79.

Zimmer, G. (1984) Fluidity of cell membranes in the presence of some drugs and inhibitors. In: *Biomembranes*, pp. 169–203. Eds. M. Kates and L. A. Manson. Plenum, New York.

Zimmer, G. and Freisleben, H. J. (1988) Membrane fluidity determinations from viscosity. In: *Advances in Membrane Fluidity*, Vol. 1, pp. 279–318. Eds. C. C. Curtain and L. M. Gordon. Alan R. Liss, New York.