Free radicals in brain, functions and failures

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Free radicals, you can't live with them and you can't live without them. Appreciation is growing that free radicals and related oxidants are ubiquitous and that they are involved in common pathways that lead to tissue damage from a wide variety of insults. Some combination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are implicated in an array of neuro-degenerative diseases (including amyotrophic lateral sclerosis and Parkinson's disease) and in many other human health problems ranging from the toxicity of some xenobiotics to cataractogenesis, carcinogenesis, atherosclerosis, diabetes mellitus, ischemia/reperfusion injury, cancer, and the aging process itself. This has created the impression that all free radicals are highly reactive and highly damaging to biological systems—in short, all bad. But a more informed examination of the chemistry of free radicals and related oxidants reveals a wide range of unique advantages they may have as regulators of normal physiological functions and as components in information processing in the brain. Furthermore, some ROS and RNS have biochemical properties that permit a role in intercellular communication, operating in the extracellular microenvironment, in a "parasynaptic" manner. Nitric oxide ($NO^{\bullet}$), superoxide ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) have all been demonstrated to be what was once thought of as nontraditional messengers. However, the paradigm is shifting to view them as rather common messengers (Sun and Oberley, 1996; Nathan, 2003; Garthwaite, 1991).

1. Properties of free radicals, ROS, and RNS

A free radical is any molecular species that contains one or more unpaired electrons. Free radicals are often produced by oxidation/reduction reactions in which there is a transfer of only one electron, or when a covalent bond is homolytically broken, that is, one electron from the pair of electrons making the bond goes with each molecule fragment. For example,

$$H\text{-}O\text{-}O\text{-}H + \text{UV light} \longrightarrow HO^{\bullet} + {}^{\bullet}OH$$

The unpaired electron causes the free radical to be paramagnetic (i.e., influenced by a magnetic field). Free radicals are physically stable and when in isolation will not readily decay. However, because a free radical has an unpaired electron it could donate this electron to an appropriate acceptor, or it could receive an electron from an appropriate donor. Thus, free radicals can be either reducing or oxidizing; some can do both.

$$\text{Flavin radical}^{\bullet} + O_2 \longrightarrow \text{flavin} + O_2^{\bullet -}$$
$$O_2^{\bullet -} + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$
$$O_2^{\bullet -} + H^{+} \longrightarrow HO_2^{\bullet}$$
$$HO_2^{\bullet} + \text{ascorbate} \longrightarrow H_2O_2 + \text{ascorbate radical}^{\bullet}$$

In these reactions superoxide is formed by a reductive electron-transfer from a more reducing flavin radical. The riboflavin radical or a flavin radical within an enzyme such as xanthine oxidase will reduce oxygen to superoxide. Superoxide is reducing and can reduce metals, such as iron when the iron is in an appropriate ligand environment. Protonation of superoxide to form its conjugate acid results in an oxidizing radical that can initiate lipid peroxidation or perhaps react with a donor antioxidant such as ascorbate. Although another radical is formed (ascorbate radical), the ascorbate radical is quite unreactive and is readily removed without inflicting oxidative damage (Buettner, 1993).

Free radicals have a wide range of reactivities and thus a range of lifetimes. In biological systems, only a very few radicals are "stable"; an example would be the radicals produced by melanins, which can be observed for many minutes and even longer; nitric oxide is moderately stable, having a lifetime of approximately 1 ms to 1 s, while highly reactive ones, such as the hydroxyl radical ($HO^{\bullet}$), have a lifetime of only approximately 10^{-9} s. The actions of highly reactive free radicals, that is, those with very short lifetimes, are restricted in time and space (site-specific), while less reactive radicals, those with long lifetimes, can have effects far removed in time and space from the site(s) of formation (Tarr and Samson, 1992). To state the obvious, if a free radical or related oxidant is longer lived, it is because it is less reactive. This lower reactivity can be viewed as the free radical or related oxidant being more selective in its reactions, which would allow it to act as a signaling molecule.

In addition to a diversity of reactivities, ROS and RNS can have a major influence on the redox environment of cells and tissues. Thiols (R-SH) and disulfides (RSSR) are contributors to the redox environment and have regulatory functions in cells and tissues (Schafer and Buettner, 2000a). Another class of proteins, whose activity can be modulated by free radicals, are those containing paramagnetic metals. Some free radicals, for example, nitric oxide, have a high reactivity with paramagnetic metals, and thus can strongly affect —for good or for bad—the manifold functions of metal-containing proteins. In the brain there are many ways to generate free radicals as well as abundant means of neutralizing their possible damaging actions. Free radicals have properties that can result in pathological consequences; however, some free radicals are vital for the neurochemistry of normal, including higher, brain functions. The destructive aspects of free radicals are demonstrable, but the normal functional role of free radicals in brain function is more complicated and not easily demonstrable.

An overview of some of the properties of a subset of ROS/RNS that are important in biology are provided in Table 1.

Recent advances in free radical chemistry applied to physiological systems have clearly demonstrated the potential pathogenicity of ROS/RNS in the brain (Halliwell, 2001; Gilgun-Sherki et al., 2002; Lipton et al., 1998; Moosmann and Behl, 2002; Koutsilieri et al., 2002). The large number of diseases and traumas in which free radicals are implicated led to the idea that free radicals are responsible not only for the pathogenesis of many diseases but also for a variety of health problems. For example, endogenously generated oxygen free radicals make about 10,000 oxidative interactions with DNA per human cell per day, and an undetermined number with proteins, lipids, and other cellular constituents. Evidence suggests that lipid peroxidation is continuous in healthy people, resulting in substantial membrane damage. However, this damage to DNA, membranes, and proteins is constantly being repaired. It is widely accepted that the accumulated products of free radical reactions are a major component in the aging process (Floyd and Hensley, 2002; Packer et al., 1997; Ames et al., 1993; Emerit and Chance, 1992).

Under normal conditions the damaging effects of free radicals are minimized by abundant protective and repair mechanisms. Dietary sources such as vegetables and fruits have a broad spectrum of "antioxidants" (e.g., carotenoids, vitamins) and the list of identified substances continues to grow. Endogenous systems include antioxidant enzymes (e.g., SOD, catalase); redox active molecules (e.g., glutathione, thioredoxin, lipoic acid); and even certain regulatory systems. We require a wide variety of antioxidants because each has its strength and weakness. They all appear to work in concert. Table 2 lists a subset of antioxidants that are important in cells and tissue.

2. Metals and free radical oxidations

The transition metals of particular importance in biological systems are zinc, iron, and copper. Although zinc is not redox active it has an important role in the antioxidant system as a component of the antioxidant enzyme CuZnSOD and its many roles in protein structure and function (Koh, 2001). Iron and copper are tightly controlled within cells, not only to fulfill their manifold functions in metallo-proteins but also to prevent their catalysis of destructive oxidative processes. Because copper and iron are redox active they can initiate the formation of free radicals in amounts that surpass endogenous protective capacity. The importance of transition metals in oxidative damage cannot be overemphasized. The concept of site-specific attack is especially relevant to the role of transition metals in unwanted, detrimental oxidations. If highly reactive oxygen free radicals are generated in the "bulk solution" of a cell, they are apt to react with thiols such as glutathione and no real harm is done; or they could react with abundant oxidizable metabolites such as glucose. The small amounts of metabolites oxidized via this route are expendable, and indeed their importance in neutralizing oxygen radicals may not be adequately recognized. Highly reactive species tend to be neutralized by functionally unimportant targets. On the other hand metals are always bound loosely or tightly to something in cells. If the metal is bound to DNA or a critical enzyme site, then the hydroxyl radical or other oxidizing species will be generated at this site and will have a very high probability of reacting with it and thereby inflict specific damage, that is, site-specific attack. To be effective, scavengers must compete for the HOo within the "cage" surrounding the bound metal. This explains why metal chelating compounds are often protective, that is, they pull these dangerous metals away from these sensitive sites. In metal-catalyzed, site-specific reactions, HOo is of most concern for inflicting cellular damage and thereby disrupting normal function, especially when generated at important targets. The current paradigm is that HOo arises from the Fenton reaction.

$$Fe^{2+} + H_2O_2 \longrightarrow HO^{\bullet} + OH^{-} + Fe^{3+}$$

However, it is becoming increasingly apparent that at physiological pH all that is needed to initiate detrimental oxidations is loosely bound Fe^{2+} and O_2 (Qian and Buettner, 1999). This combination leads to oxidants that will initiate free radical formation in cells (Schafer et al., 2000). In addition, this loosely bound ferrous iron is more damaging at lower pH values such as those found at sites of inflammation or in lysosomes (Schafer and Buettner, 2000b). Thus, iron is very well managed, keeping in coordination environments that do not allow unwanted oxidative catalysis to occur (Miller et al., 1990).

$$Fe^{2+} + O_2 \longrightarrow \text{damaging oxidants}$$

Cell injury by almost any means may result in conditions that in turn release catalytic metals. The dysregulation of these redox active metals is thought to be the basis of some pathologies. Consider the following examples

- Free radicals produced by the catalytic action of ferrous ions in a "reactive state" may contribute substantially to the damage of substantia nigra neurons in Parkinson's disease (Olanow et al., 1992).
- The transition metals iron, copper, and zinc have been implicated as key mediating factors in the pathophysiology of Alzheimer's disease (Cuajungco and Faget, 2003)
- Copper-binding proteins play important roles in disorders with neurological symptoms (Menkes and Wilson disease) and neurodegenerative diseases such as Alzheimer's. The Menkes and Wilson proteins are copper transporters, while the amyloid precursor protein can transport copper or zinc (Strausak et al., 2001).
- Beta-amyloid has been proposed to be a preventive antioxidant for brain lipoproteins by chelating transition metal ions. However, accumulation of beta-amyloid lipoprotein aggregates could lead to production of ROS and subsequently neurotoxicity (Kontush, 2001).
- Beta-amyloid (Abeta) can react with excess brain metal to form metal-enriched precipitates (plaques) (Bush, 2002). The reaction of Abeta with copper or iron produces hydrogen peroxide that could mediate the oxidative damage seen in brain of Alzheimer's patients. Increased lipid peroxidation products (4-hydroxy-2- nonenal, and acrolein, both reactive aldehydes) have been found in the brain, cerebrospinal fluid, and plasma of Alzheimer's patients (Arlt et al., 2002).
- Beta-amyloid may have two contrasting roles: as a metal chelating antioxidant and as a pro-oxidant when aggregated in brain tissue (Kontush, 2001). (See previous point.)
- Wilson disease is a copper-metabolism disorder. Malfunctioning of a coppertransporting ATPase results in toxic accumulation of copper in liver and brain (Fatemi and Sarkar, 2002).
- Prion disease is a neurodegenerative disorder in which prion protein (PrP) undergoes post-translational conformation change. The PrP is thought to be a copper-binding antioxidant. It can bind other metals such as zinc and manganese with lower affinity. In prion disease it is proposed that PrP binds not only copper but also zinc, resulting in a loss of its antioxidant function (Wong et al., 2001)

3. Free radicals and iron-sulfur clusters in regulatory proteins

Iron-sulfur (Fe-S) clusters are constituents in several hundred known proteins that have regulatory significance. Owing to the redox reactivity of iron and its flexible coordination chemistry, these clusters have essential roles in a variety of enzymatic reactions. A mammalian example of an Fe-S cluster-containing enzyme is aconitase. In mitochondria, aconitase converts citrate to isocitrate in the tricarboxylic acid cycle. However, superoxide can react with the Fe-S cluster inactivating the enzyme. When the Fe-S cluster of cytosolic aconitase is inactive the enzyme becomes an ironregulated RNA-binding protein. Thus, Fe-S clusters may be significant molecular switches in a variety of regulatory systems, and agents such as $NO^{\bullet}$ or $O2^{\bullet}$ can "throw the switch" by reacting with the iron (Haile et al., 1992).

4. Free radicals in DNA synthesis, ribonucleotide reductase

Free radicals have an essential, normal mechanistic role in DNA synthesis. The enzyme ribonucleotide reductase (RNR) catalyzes the rate-determining step of DNA biosynthesis. These enzymes bring about the conversion of nucleotides to deoxynucleotides. There are at least three classes of RNRs and each initiates the conversion by formation of a protein free radical

- Class I: diferric tyrosyl radical
- Class II: adenosyl cobalamin free radical (carbon-centered)
- Class III: glycyl radical

Each of these radicals then initiate the formation of a cysteinyl radical on the protein backbone (Figure 1). This radical is common to all forms of RNR. This thiyl radical then abstracts a hydrogen atom from ribose to bring about the formation of deoxyribose. This is an example of an emerging picture of how free radicals are involved in the fundamental biochemistry of life

5. Sources of free radicals in the brain

There are many ongoing sources of free radicals in the brain (Halliwell, 1992; Esposito et al., 2002). Mitochondrial respiratory processes generate $O2^{\bullet\cdot}$, and when the free radicals produced overwhelm the endogenous antioxidants, the resultant oxidants are harmful to DNA, membrane lipids, and proteins. Indeed, free radicals are implicated in many of a growing list of "mitochondrial diseases." Bruce Ames and his colleagues argue that oxidants generated by mitochondria are the major source of oxidative lesions accumulated with age (Head et al., 2002; Liu et al., 2002). A number of enzymes generate free radicals: xanthine oxidase, cytochrome P450, monoamine oxidase, nitric oxide synthase, NADPH-oxidases are only a few examples.

Sources of free radicals are also found in the extracellular fluid resulting from, for example, the auto-oxidation of the catecholamines, norepinephrine and dopamine. The autoxidation of catechols to quinones generates reduced forms of molecular oxygen, for example, superoxide and H_2O_2. Metabolic pathways related to the eicosanoids (e.g., arachidonate) that involve the cyclo-oxygenase or lypoxygenase enzymes are another major source (Keyser and Alger, 1990). Glutamate activation of the N-methyl-D-asparate (NMDA) receptor sets off both an arachidonic acid cascade in striated neurons and increased $NO^{\bullet}$ synthase activity. Doubtless many other generators have not yet

been identified.

6. Free radicals and pathology

The brain is susceptible to free radical damage because it has a high concentration of polyunsaturated fatty acids, is relatively low in antioxidant capacity, has a high rate of oxygen use, and has a high concentration of transition metals (e.g., nonheme iron) in some regions. When free radical—mediated lipid peroxidation is initiated, lipid hydroperoxides are generated and the membrane properties are altered (Ong and Packer, 1992). Membranes tend to become less fluid, more leaky to ions, and membrane-bound receptors may be affected. The products of free radical—mediated chain reactions of lipid peroxidation are complex. In addition to the free radical—mediated changes, the aldehydes produced are especially toxic. In particular, the 4-hydroxyalkenals can react with DNA, inhibit enzymes, and inactivate receptors. Although evidence is abundant that highly reactive free radicals cause irreversible pathology when tissues, cells, or cellular constituents are exposed to levels of radicals that exceed the protective and repair systems' capabilities, direct evidence that they are the "final destructive agents" in a long list of disorders is difficult to gather. Damage in some cases, for example, following radiation exposure, is clearly free radical-mediated. But in the neurodegenerative diseases or pathology following head trauma, multiple events are detrimental and the extent to which the radicals contribute is difficult to dissect. Direct detection of ROS/RNS in vivo is a nearly impossible challenge. Their presence is inferred by products generated ("foot prints") or by changes in exogenous chemical indicators, which provide only indirect evidence. A demonstrated imbalance in the antioxidant network in a disease condition (e.g., abnormal enzyme levels) is strongly suggestive of free radical involvement; but some puzzling results have been reported. For example, superoxide dismutase (SOD) is a major defense against oxidative tissue damage, yet an overabundance of SOD can be toxic (Zhang et al., 2002).

The gene for CuZnSOD is located on chromosome 21. This location results in an overexpression of CuZnSOD. It has been observed that intelligence of Down syndrome patients is directly related to the level of glutathione peroxidase, an enzyme that removes hydrogen peroxide formed by SOD. This is a key observation, demonstrating that an appropriate balance in the antioxidant network is needed for optimal health.

7. Nitric oxide, a multi tasking molecule

Nitric oxide (nitrogen monoxide, $NO^{\bullet}$), an essential yet potentially toxic molecule, has a wide range of biological functions, including major roles in higher brain functions (Beckman et al., 1993). The primary partners in the reaction of nitric oxide are oxygen, superoxide, thiols, and redox metals. Some important reactions of $NO^{\bullet}$ in biology are with the following (Halliwell and Gutteridge, 1999):

- O_2: oxidizes $NO^{\bullet}$ to nitrite; this is thought to be a very minor process in biology because of the many other choices of reaction partners available.
- $O_2^{\bullet\cdot\cdot}$: produces peroxynitrite (ONOO- and ONOOH). These species are very reactive and damage proteins by introducing $-NO_2$ onto the tyrosine ring (nitration of tyrosine); it also reacts with DNA. High levels of nitration are seen in many pathologies where both $NO^{\bullet}$ and $O_2^{\bullet\cdot\cdot}$ are made at inappropriately high levels.
- -SH (Thiols): with the aid of transition metals $NO^{\bullet}$ can react with thiols to form nitroso thiols (-SNO). Some researchers view these species as a "storage" form of nitric oxide. Because the effective lifetime of $NO^{\bullet}$ is increased, its action will be different in both time and space
- Ferrous-hemes: $NO^{\bullet}$ coordinates tightly to these compounds. For example it coordinates to the iron of guanylate cyclase, thereby activating it to synthesize cGMP, a well-known intracellular signal
- Fe-S clusters: they are essential for the function of various enzymes. Reaction of $NO\cdot$ with Fe-S clusters changes the activity of these enzymes
- Oxyhemoglobin: reacts with $NO^{\bullet}$, producing nitrate (NO_{3-}) and methemoglobin. This is thought to be a major route for the removal of $NO^{\bullet}$.
- Cytochrome oxidase: this is a key enzyme in mitochondrial respiration. NOo binds reversibly to the ferrous heme in an equilibrium reaction, thereby regulating the fundamental metabolism of the cell
- Peroxyl radical: NOo reacts rapidly with peroxyl radicals, such as those formed during free radical-mediated lipid peroxidation, thereby acting as a very effective antioxidant

Because glutamate activation of the NMDA receptor leads to the synthesis of $NO^{\bullet}$, the inappropriate conversion of $NO^{\bullet}$ to peroxynitrite may be the linkage between glutamate and free radical-induced damage. Many pathophysiological processes including ischemia-reperfusion could initiate the simultaneous production of $NO^{\bullet}$ and $O_2^{\bullet\cdot\cdot}$. Inhibitors of nitric oxide synthesis have been shown to reduce the infarct volume in a rodent model of cerebral ischemia. It appears that the neurotoxicity of $NO\cdot$ results from its reaction with superoxide forming

peroxynitrite (Figure 2), whereas the neuroprotective effects may result from down regulation of NMDA receptor activity by reaction with thiol groups of the receptor's redox modulatory domain. It is clear that $NO^{\bullet}$; does not act like traditional neurotransmitters: it is not stored in vesicles, its known targets are not membranebound receptors, it does not follow a simple, one-way conduction across synapses, and it can be highly toxic (Smythies, 1999; Gilgun-Sherki et al., 2001; Garthwaite, 1991).

8. Thiols, disulfides and redox environment

Cells and tissues must maintain a reducing environment to survive. This reducing environment provides the electrochemical gradient needed for electron flow. The movement of electrons provides the energy needed to build and maintain cellular structures and associated functions. An array of redox couples is responsible for the electron transfer. The redox environment of a cell is a reflection of the state of these couples. It is now realized that the biological state of the cell changes with the reducing environment of the cell (Schafer and Buettner, 2000a). Many transcription factors are sensitive to redox changes in the cell. Thus, changes in the cellular redox environment can initiate signaling cascades (Sun and Oberley, 1996). The signaling by redox mechanisms relies on the chemistry driven by changes in the electrochemical potential of redox couples in the cell. Two of the most important redox couples in free radical and redox biology are the glutathione system (GSSG/2GSH) and the nicotinamide adenine dinucleotide phosphate system ($NADP^+$/NADPH). Glutathione is considered the major thioldisulfide redox buffer of the cell (Gilbert, 1990). NADPH is a major source of electrons for reductive biosynthesis and the source of electrons for the maintenance of the glutathione system (Figure 3).

The redox couples in cells and tissues can be viewed as electrochemical cells. The Nernst equation allows the determination of the voltage of an electrochemical cell (ΔE) and the reduction potential of the two partners in an electrochemical cell. The reduction potential of one partner is referred to as the electrochemical half-cell reduction potential, Ehc.
In case of the GSSG, $2H^+$/2GSH couple, the reduction half-reaction for this couple is

$$GSSG + 2H^+ + 2e^- \longrightarrow 2GSH$$

Note that one molecule of GSSG forms two molecules of GSH. Therefore, [GSH] will enter the Nernst equation as a squared term

$$E_{hc} = -240 - \frac{59.1}{2} \log \frac{[GSH]^2}{[GSSG]} \quad \text{mV at 25°C, pH 7.0}$$

The value of Ehc for the GSSG, $2H^+$/2GSH couple in cells and tissues is negative, consistent with a reducing environment. It has been observed that in the healthy brains of young mice, this value is around -220 mV; however, when subjected to an oxidative stress Ehc becomes more positive. Using *tert*-butyl hydroperoxide as a transient oxidative stress, Adams et al. (2001) found that Ehc increased to -150 mV, and was restored to normal values in less than 2 hours.

Changes in brain redox environment can alter the equilibrium of many thiol disulfide couples (e.g., proteins containing -SH groups) in the cell, triggering signaling events by redox mechanisms. Some examples of thiol disulfide exchange reactions are

$$PSH + GSSG \leftrightarrow PS\text{-}SG + GSH$$
$$2GSH + PS\text{-}SP \leftrightarrow 2PSH + GSSG$$
$$P(S\text{-}S) + 2GSH \leftrightarrow P(SH)_2 + GSSG$$

where PSH and P(SH)2 are protein thiols; PS-SG and P(S-S) are a mixed and an intramolecular disulfide (thiols are oxidized to form disulfides).These types of reactions result in metabolic changes as well as changes in signal transduction cascades.

A number of ligand-gated receptor-channel complexes have functionally important disulfide bonds that endow these proteins with the ability to undergo conformational changes. Changes in the cellular redox environment will influence the transduction of information across membranes by changing the behavior of such receptors. The nicotinic acetylcholine receptor is a classic example of chemical modification of a disulfide bond's altering the receptor function. Both the channel gating and conductance of the nicotinic receptor at the neuromuscular junction are affected by the state of the extracellular disulfide bond. The dopamine D1 receptor has a thiol group that is essential for ligand binding and represents a potential redox modulator domain. The ligand recognition site of the 5-hydroxytryptamine receptor in the cortex and hippocampus is affected by the state of thiol/disulfide groups. The hydrophilic extracellular domains have an important, previously unexpected role in the ligand-binding site of the $ß_2$-adrenergic receptor. By site-directed mutagenesis, HG Dohlman and coworkers (1990) identified four cysteines critical for normal ligand-binding affinities and for the proper expression of a functional $ß_2$-adrenergic receptor at the cell surface. Furthermore, these four cysteines are in the extracellular hydrophilic loops connecting the transmembrane segments.

Opiate receptor responsiveness may be modified in vivo through changes in redox environment, providing a physiological mechanism for modulating nociception. Thiols are involved in the binding of opiates to brain membranes and may differentially affect the binding of agonists and antagonists. For example, Cu^{2+} given into the cerebral ventricle of mice induces analgesia that can be reversed by the thiol

reductant dithiothreitol; also, low doses of dithiothreitol have antagonized the analgesia induced by morphine sulfate. Both sulfhydryl and disulfide groups are specifically involved in the molecular processes mediating the aggregation of the ligand-receptor complexes. These examples demonstrate that changes in the disulfide-thiol redox state modifies receptor binding tuned to the redox status in the extracellular microenvironment.

Another example is Parkinson's disease, which involves neurodegeneration of dopaminergic neurons of the substantia nigra. It has been found that cytosolic glutathione is drastically depleted in the substantia nigra of Parkinson patients (Bharath et al., 2002). This may well be connected to dysregulation of metals. One more example is glutamine, a precursor for the neurotransmitter glutamate. Glutamate is required for the biosynthesis of glutathione. Thus glutamine is important in the regulation of the cellular redox environment that can influence brain metabolism, cell proliferation and cell death (Mates et al., 2002). The quantitative understanding of the redox state of redox couples such as GSSG/2GSH and $NADP^+$/NADPH are in their infancy. But it is clear that the redox environment of cells and tissues control the biological function and overall health of the organism.

9. Redox modulation of the NMDA receptor

Excitatory transmission in the brain is largely mediated by the neurotransmitter glutamate, and the NMDA receptor is a major member of the glutamate receptor family. These receptors are indispensable in information processing related to neural plasticity, memory, and learning. A novel modulatory site on the NMDA receptor sensitive to sulfhydryl reagents has been described. The sequence of the NMDA receptor reveals several cysteine residues in the proposed extracellular domain (Moriyashi et al., 1991). The NMDA receptor is inhibited by some reductants (ascorbate, hydroquinone), while others (dithiothreitol, mercaptoethanol) potentiate its function (Tang and Aizenman, 1993). The different results from the various reductants may be related to their accessibility to the thiol groups. Further, the "reduction-mediated" inhibition of the NMDA receptors may be related to the resistance to NMDA-mediated injury of neurons containing high levels of NADPH-diaphorase. Lipton (1999) { reported that the NMDA-evoked increases in $[Ca^{2+}]i$ and whole cell current of rat cortical neurons are inhibited by $NO^{\bullet}$, indicating that nitric oxide down-regulates NMDA receptor activity via a redox modulatory site. Thus, the NMDA receptor, a receptor directly involved in both higher brain functions and in excitotoxicity, contains redox-sensitive regulatory centers. The redox sensitive pairs of cysteine residues are exposed to, and influenced by, the redox environment of the extracellular milieu.

Free radicals can also influence the level of extracellular glutamate by inhibiting glutamate uptake by astrocytes. The extracellular glutamate increase could facilitate normal excitatory events or, on the other hand, be excitotoxic if increased beyond some threshold.

10. Learning and memory

The cellular basis for learning and memory is, in part, the facilitation of the sending and receiving of signals of neurons to one another. An enduring change in synaptic efficacy in mammalian brain, termed long-term potentiation (LTP), is widely accepted as one neural component in learning and memory. For LTP to develop, a "retrograde signal" must travel backward (i.e., from post- to presynaptic) to enhance the presynaptic transmitter release. Nitric oxide and carbon monoxide, either alone or in combination, probably serve as retrograde signals. Hydrogen peroxide blocks LTP in rat hippocampal slices, suggesting that a reactive oxygen intermediate can selectively modify synaptic mechanisms in the hippocampus.

Another potential retrograde signal is arachidonate, a membrane lipid present both in physiological and pathophysiological processes. Oxygen free radicals originate from arachidonate metabolism by way of cyclooxygenase and lipoxygenase pathways, but the importance of radicals in the signaling is not clear. Keyser and Alger (1990) found that arachidonate depressed the calcium channel currents in hippocampal pyramidal cells; the researchers suggested that the modulating action of arachidonate acid was mediated via protein kinase C activation and the generation of free radicals.

11. Conclusions

Free radicals may have a "double life," not only as substances that can be devastating to brain cells but also with vital cell functions. A more detailed understanding of this duplicity is emerging as a topic of considerable importance. Free radicals have a wide range of reduction potentials; they can be either oxidizing or reducing, and they have a wide spectrum of lifetimes. Thus, some could diffuse into the extracellular environment and affect remote targets, while others are time limited and space limited and thereby "site-specific" in their reactions. New methods and techniques are continuously being developed that will allow us to improve our understanding of the role of ROS/RNS in disease.

Free radicals are ubiquitous in biological systems. Evolutionary selection has resulted in biochemical and cellular mechanisms for exploiting their usefulness while necessarily dealing with their toxicity. However, on the long haul, that is, aging and age-related neurodegenerative diseases, the endogenous protectors lose.

It appears we are now emerging from a one-way transmitter-centered era of neurophysiology and are embarking on an historic period of discovery of the double life of ROS/RNS as molecules subserving brain functions, yet at the same time exacting a price of ultimate brain failure. Along with the many known kinds of molecular regulators, free radicals possess properties that provide a high degree of plasticity and versatility required for the neurochemical bases of higher brain functions, as in learning, memory, and cognition.

12. See also

Aging of the brain
Nitric oxide in the nervous system
NMDA receptors
Neuroprotection

13. References

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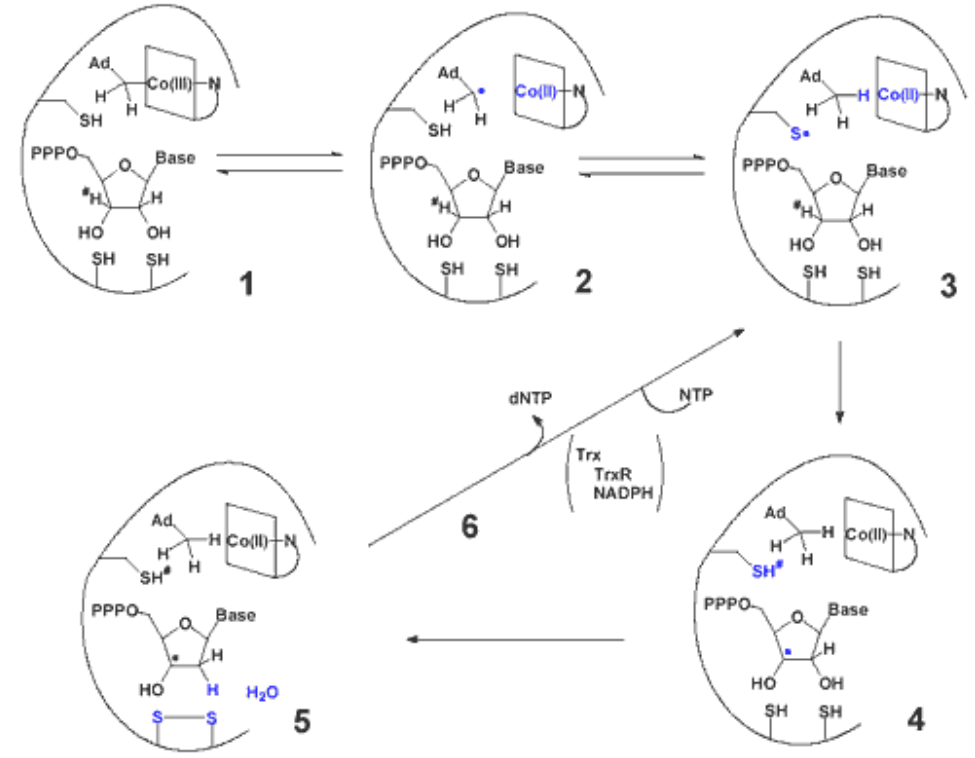

Figure 1. This illustration represents current thinking on the mechanism of Class III ribonucleotide reductase (RNR) (Stubbe and van der Donk, 1998; Licht et al., 1999). The bond energy of the carbon-cobalt bond is only about 30 kcal mol^{-1}; thus visible light can cleave the bond. This is an example of an emerging picture of how free radicals are involved in the fundamental biochemistry of life.

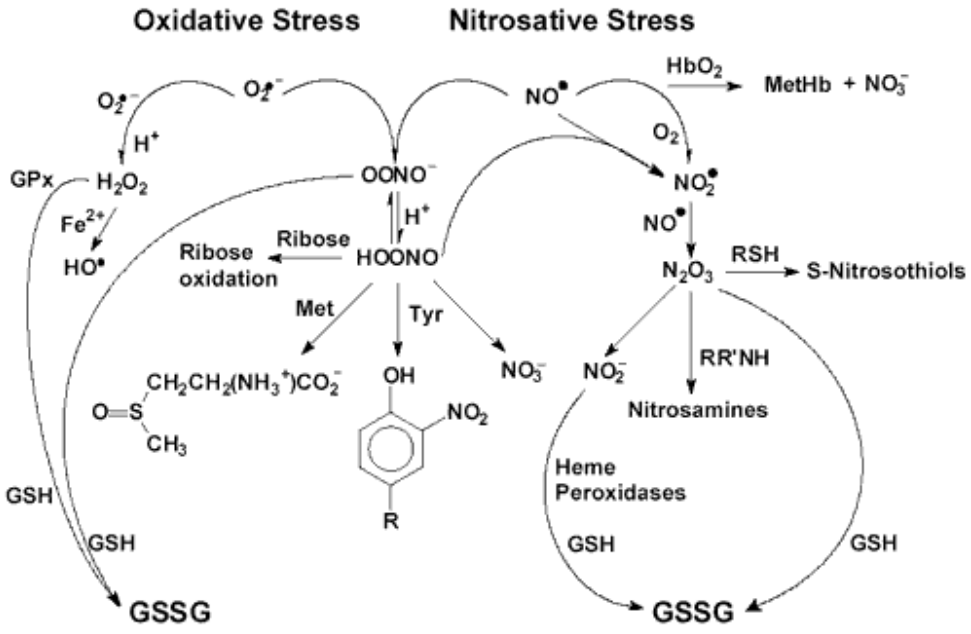

Figure 2. Oxidative and nitrosative stress. This scheme shows how various ROS and RNS are formed and react

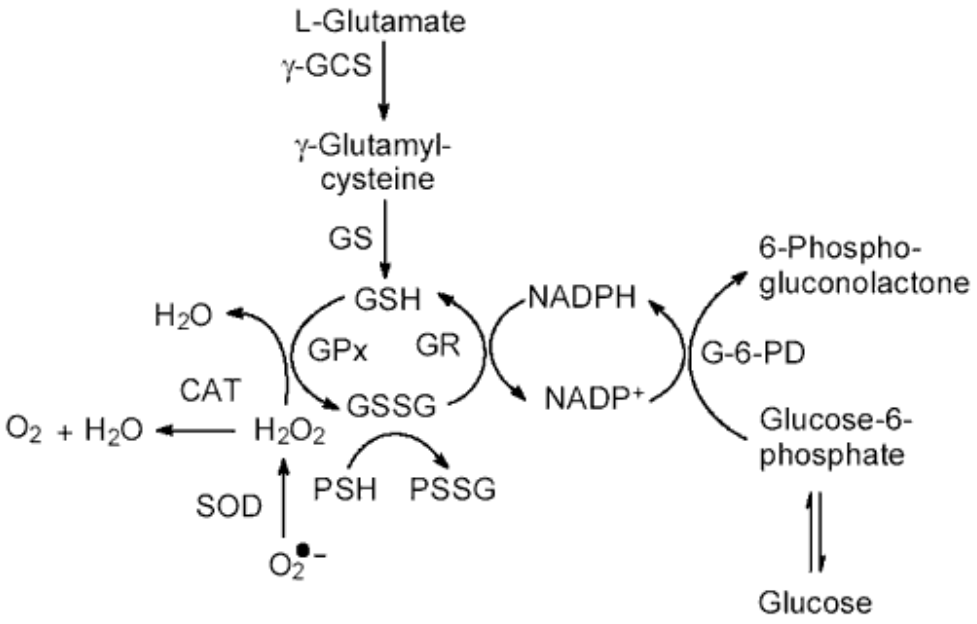

Figure 3. Part of the antioxidant network. This schematic shows the relationships between antioxidant enzymes and small molecule antioxidants such as glutathione and NADPH. Abbreviations: CAT = catalase; G-6-PD = glucose-6-phosphate dehydrogenase; γ-GCS = γ-glutamylcysteine synthetase; GS = glutathione synthetase; GPx = glutathione peroxidase; GR = glutathione reductase; PSH = protein thiols; PSSG = mixed protein disulfide; SOD = superoxide dismutase

Table 1. Common ROS/RNS

Name	Chemical Formula! notation	Characteristics
Hydroxyl radical	$HO^{\bullet}$	The most oxidizing free radical formed in a biological setting. Nearly always damaging. Short-lived, reacts immediately, life time in biology $\approx 10^{-9}$s
Superoxide	$O_2^{\bullet -}$	Formed in mitochondria and by enzymes. Phagocytic cells make lots for host defense; is converted to H_2O_2. Somewhat reactive, weak reductant. Removed by SOD.
Hydroperoxyl radical	$HO_2^{\bullet}$	Protonated superoxide, $pK_a = 4.7$; oxidizing; can initiate lipid peroxidation. Dismutes forming H_2O_2 and O_2.
Peroxyl radical	$ROO^{\bullet}$	Oxidizing; formed during free radical-mediated lipid and protein oxidation.
Alkoxyl radical	$RO^{\bullet}$	More oxidizing than peroxyl radicals. Formed during free radical-mediated oxidations.
Singlet oxygen	$^1O_2$	Formed by light in skin and by photo sensitizers; oxygen with extra energy; oxidizing molecule.

Hydrogen peroxide	H_2O_2	Formed from superoxide and by enzymes. Important in controlling cellular redox environment, but damaging and toxic at high levels.
Hydroperoxide	ROOH	Formed in lipids and proteins during free radical oxidations. Can lead to more damage. Removed by enzymes such as GPx.
Hypochlorous acid	HOCl	Formed by macrophages from H_2O_2 and Cl^- by myeloperoxidase. Important in host defense. Is oxidizing.
Aldehydes	-CHO	Although not thought of traditionally as ROS, they are formed upon oxidation of lipids and proteins. They react with protein and DNA, producing "damage" .
Nitrogen monoxide, nitric oxide	$NO^{\bullet}$	Long-lived; diffuses over many cell diameters; reacts with metalloproteins in signaling; an excellent antioxidant.
Peroxynitrite	$ONOO^-$	Formed by reaction of $NO^{\bullet} + O_2^{\bullet -}$; highly oxidizing; nitrates tyrosine in proteins (adds $-NO_2$ to the ring).
Peroxynitrous acid	ONOOH	pK_a = 6.8; the acid form of $ONOO^-$; is an oxidant; see above.
Nitrogen dioxide	$NO_2^{\bullet}$	An oxidizing radical
Dinitrogen trioxide	N_2O_3	Oxidant and nitro sating agent; removed by reaction with water to yield nitrite.

Table 2. Common Antioxidants*

Antioxidant	**Common Abbreviation Properties**	**Properties**
Ascorbate (Vitamin C)	$AscH^-$	Water soluble; donates electron/hydrogen atom to oxidizing radicals.
Tocopherols (Vitamin E)	TOH	A family of oil (membrane) soluble antioxidants that protect lipids in mem branes and LDL from free radical oxidations. α-Tocopherol is the most abundant form in humans. Donates electron/hydrogen atom to oxidizing radicals.
Beta-carotene	ß-car	Oil soluble; protects against singlet oxygen; may provide some protection against radicals.
Flavonoids and related polyphenols		Nutritional antioxidants that support health by unknown mechanisms.
glutathione	GSH	Endogenously produced sulfhydryl (thiol) containing antioxidant. A cofactor for enzymes that remove peroxides.
Nitric oxide	$NO^{\bullet}$	Chain breaking antioxidant; very effective against lipid peroxidation. Works together with vitamin E.

Superoxide dismutases	SOD	A family of enzymes that remove superoxide, converting it to hydrogen peroxide and oxygen.
Catalase	Cat	Removes hydrogen peroxide, converting it to water and oxygen.
Glutathione peroxidases	GPx	A family of enzymes that removes hydroperoxides, both organic hydroperoxides and H_2O_2. Requires GSH to function. Contain selenium, which is essential for activity.
Peroxiredoxins	Prx	A family of enzymes that remove H_2O_2; key in signaling during oxidative stress.
Alpha-Lipoic acid	α-LpA	An antioxidant with two thiols groups; is able to recycle vitamins C and E, glutathione and Coenzyme Q; some consider it to be the antioxidants' antioxidant.
Coenzyme Q	CoQ or CoQ_{10}	A key component of the mitochondrial electron transport chain, but also an excellent fatsoluble antioxidant; also able to recycle vitamin E

* This table lists a subset of antioxidants. These antioxidants may have biochemical functions in addition to their action as antioxidants.