Review Article

Mechanism of Oxidative Stress in Neurodegeneration

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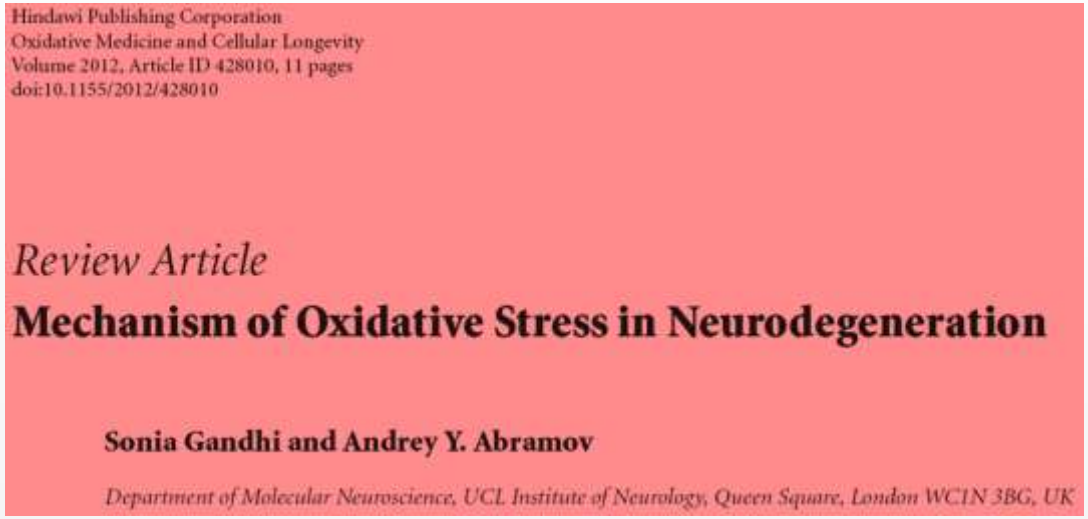

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OXIDATIVE STRESS & NEURODEGENERATION

Oxidative Stress & Neurodegeneration

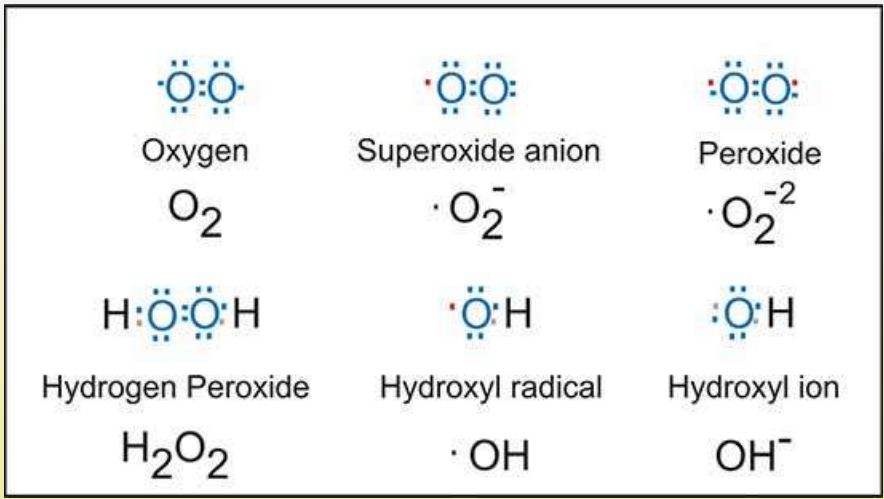
- Oxidative stress is important in their etiology (association)
- **Aging** has been established as the most important risk factor (AD & PD)
- **Aging: cumulative oxidative stress** leads to mitochondrial mutations, mitochondrial dysfunction, & oxidative damage
- Is oxidative stress a result of dysfunctional & dying neurons? or
- Does oxidative stress itself cause the dysfunctionality/death of neurons?
- How does a global event such as oxidative stress result in the selective neuronal vulnerability seen in most neurodegenerative diseases?
- & finally, if oxidative stress is truly fundamental to pathogenesis, then will the use of antioxidant therapy be successful?

OUTLINE

- In order to address these questions:
  - Definition of oxidative stress
  - Show how ROS is generated in the human brain
  - The antioxidant defense mechanisms
  - Is there an evidence that oxidative stress can be found in neurodegenerative disease?
  - Is oxidative stress truly pathogenic in disease models?
  - What treatment experimental studies have been performed?

Oxygen, Brain & Oxidative Stress

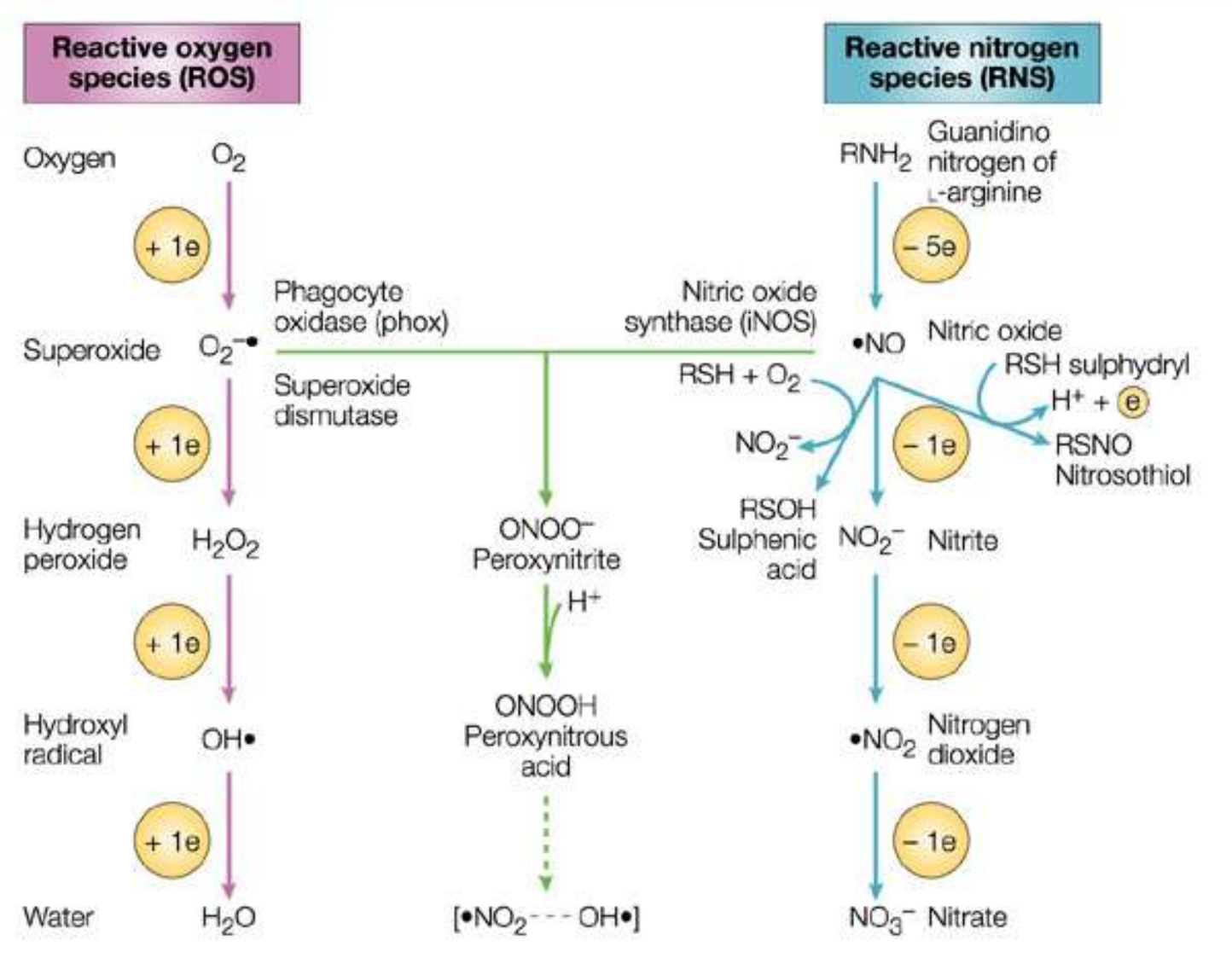
- **Oxygen is essential** for the normal function (respiration, high redox potential, excellent oxidizing agent)
- Neurons & astrocytes, are responsible for the massive consumption of O2 **(~2% vs. >20%)**
- The state of **hyperoxia produces toxicity** (including neurotoxicity)
- Partially reduced forms of oxygen are highly active (**ROS**)
- Varieties of (ROS): superoxide (**$O^{\bullet -2}$**), hydrogen peroxide (**H_2O_2**), & hydroxyl radical (**OH•**) (the most reactive)
- The modern use: radicals & non-radicals (**O_3, O_2, OH^-**)
- **What do they do**? Chemically interact with biological molecules
- Aerobic organisms survive its presence only because they contain **antioxidant defenses**



Oxygen, Brain & Oxidative Stress

- Brain cells require **more effective** antioxidant protection:
  - *They exhibit **higher** (10-fold) oxygen consumption*
  - ***Non-dividing** cells (long life duration)*
  - *Nitric oxide has a prominent role in the brain (**RNS**)*
- **Oxidative stress**: is a condition in which **the balance** between production of **ROS & level of antioxidants** is significantly disturbed & results in damage to cells by **excessive ROS**
- **ROS may target several different substrates** in the cell, causing **protein, DNA, RNA oxidation**, or **lipid peroxidation**

Oxygen, Brain & Oxidative Stress

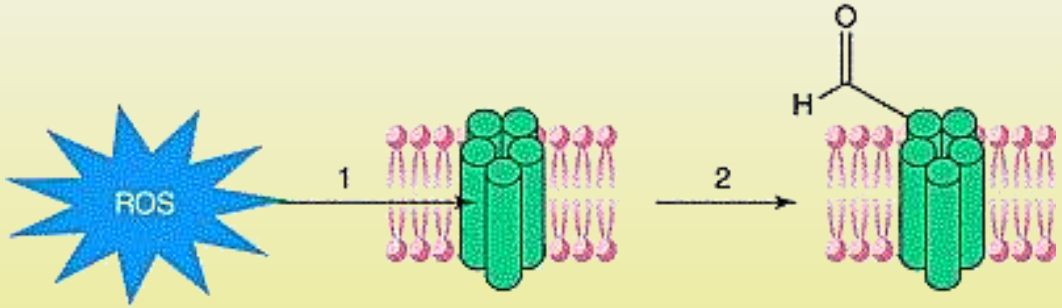


Oxygen, Brain & Oxidative Stress

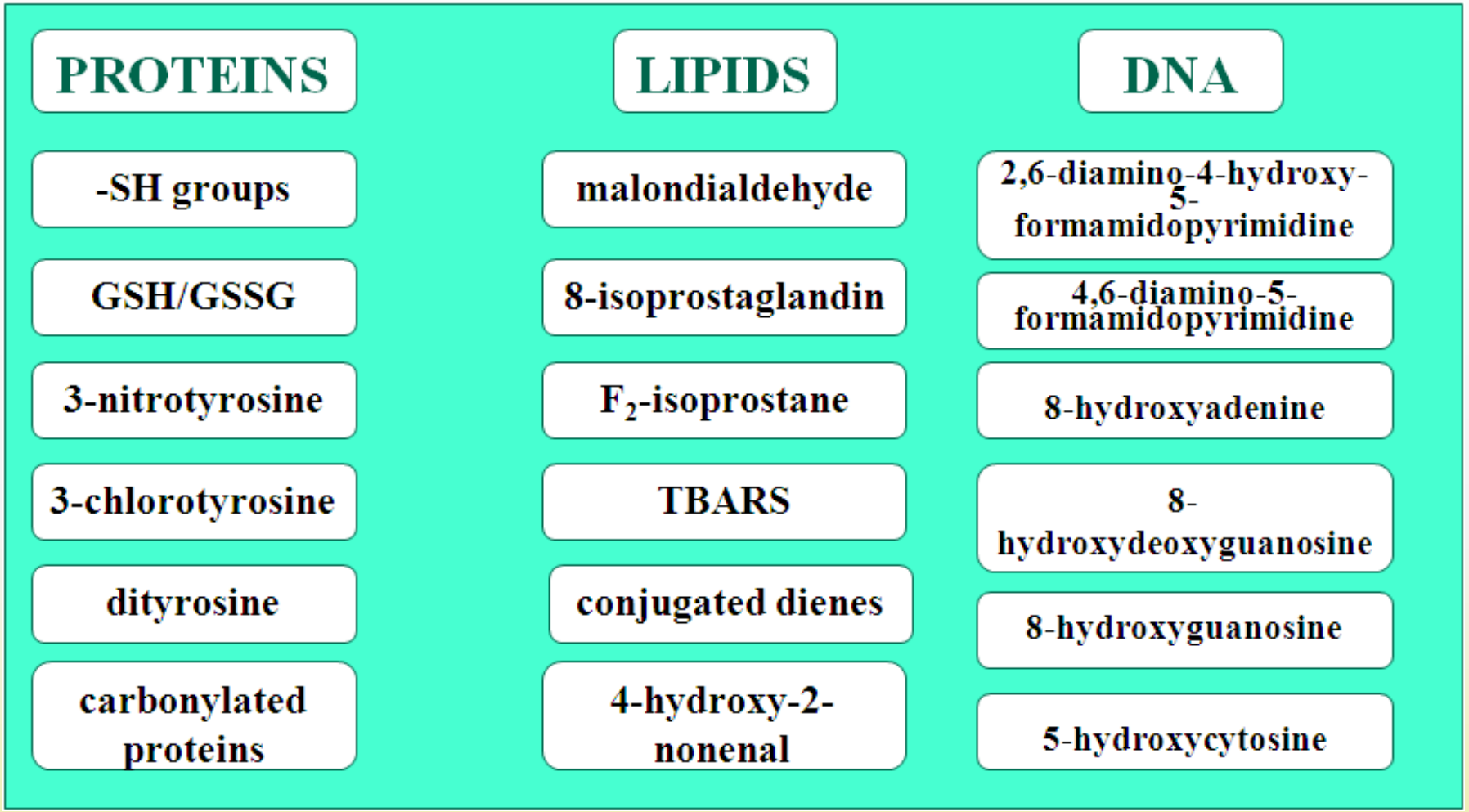
- Lipid peroxidation products of **polyunsaturated fatty acids:** especially **arachidonic acid & docosahexanoic acid (DHA)** which are abundant in brain, are **malondialdehyde & 4-hydroxynonenal**

- ROS attacks **protein**, oxidizing both the backbone & the side chain, which in turn reacts with amino acid side chains to form **carbonyl functions (**oxidation can yield aldehydes and ketones)

- ROS attacks **nucleic acids** in a number of ways, causing DNA-protein crosslinks, breaks in the strand, & **modifies purine & pyrimidine bases** resulting in DNA mutations



Oxygen, Brain & Oxidative Stress

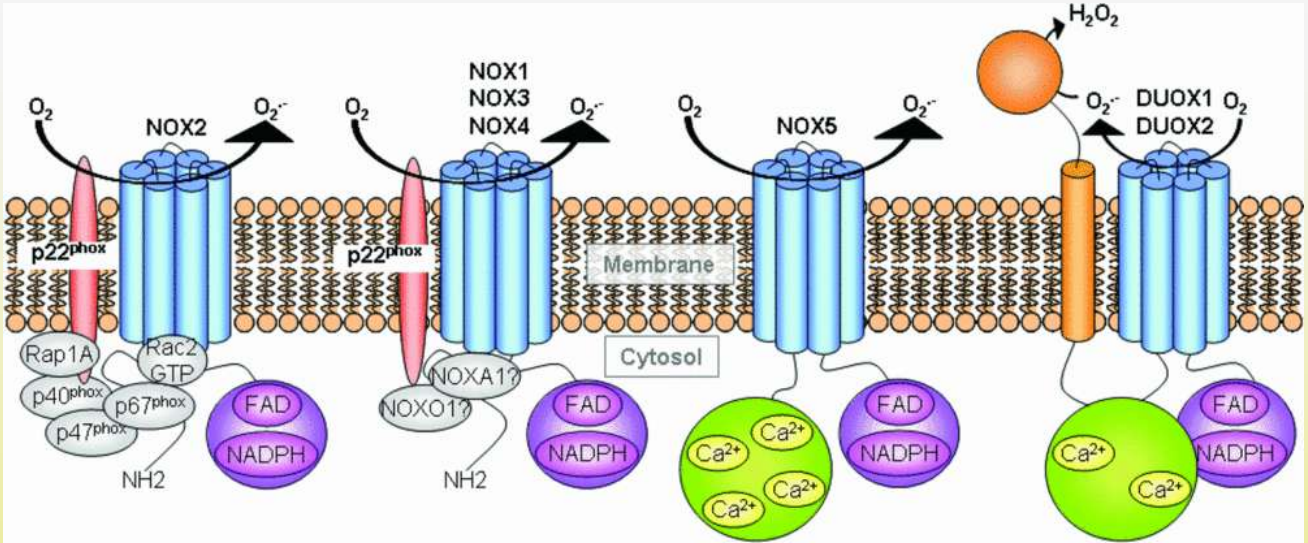


ROS PRODUCERS IN MAMMALIAN BRAIN

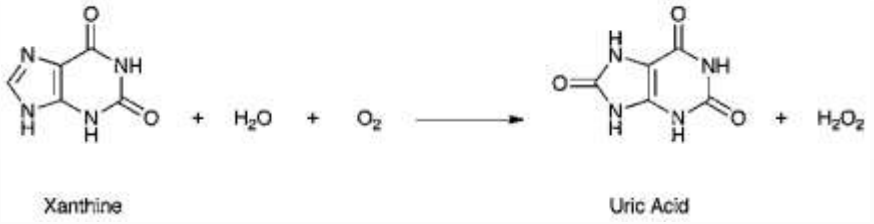
NADPH Oxidase

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet -} + NADP^+ + H^+$$

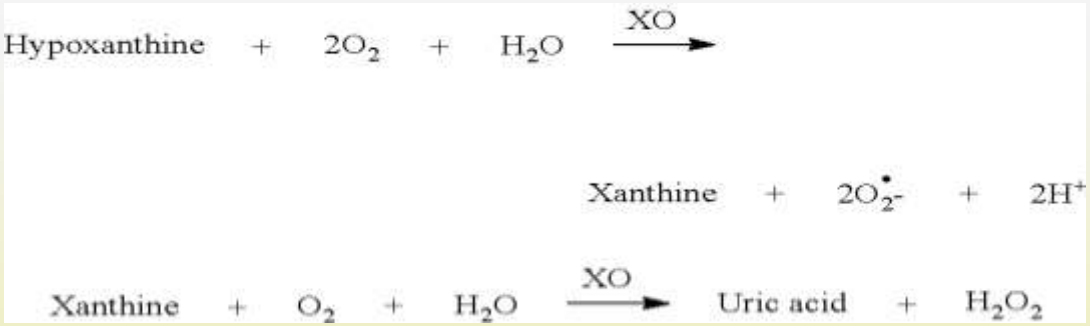
- A **multi-subunit** enzyme complex
- Is a **member of the NOX gene** family
- Also called phagocytic oxidase (**PHOX**)
- Seven NOX genes have been identified
- The most expressed of the NOX enzymes **in the brain is NOX_2**
- The enzyme transfers the proton across the membrane, & the **end product of the enzyme is superoxide**

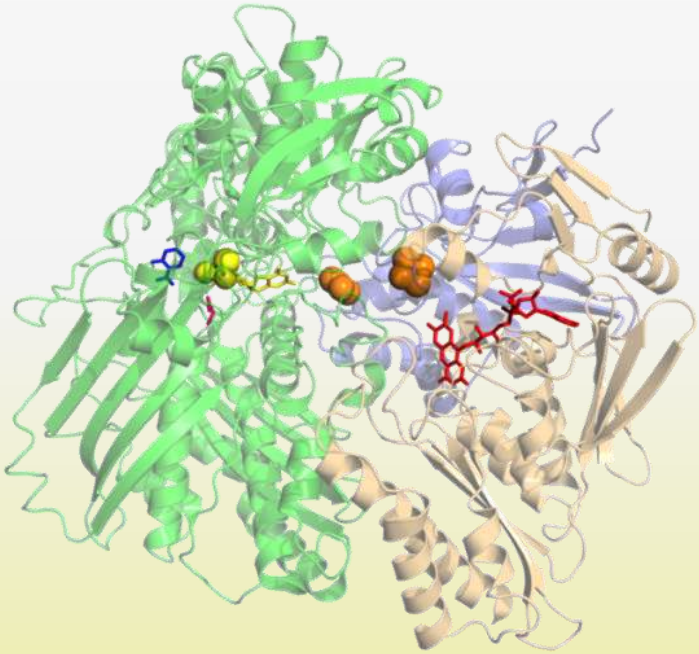


Xanthine Oxidase



- It is a **molybdo-flavo-enzyme** complex
- A key enzyme of **purine catabolism**
- XO catalyses the oxidation of a wide range of substrates & **pass electrons to molecular oxygen to produce uric acid, superoxide, & hydrogen peroxide**





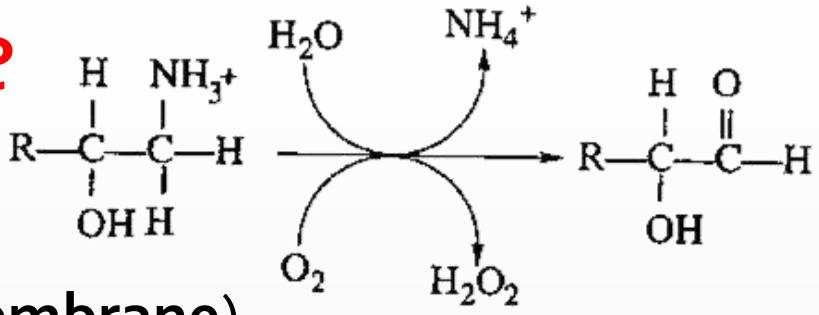
Mitochondria

- Mitochondria (electron transport chain-ETC), in contrast to other cellular producers of ROS, **generate free radicals all the time**
- Mitochondria, which harbor the bulk of oxidative pathways, **leak single electrons to oxygen**
- Depending on the metabolic conditions, isolated mitochondria produces **superoxide** in e.x.;
  - ***Respiratory complex I***
  - ***Complex III***
  - ***Aconitase***
  - ***α-ketoglutarate dehydrogenase complex***
- The production of **superoxide is dependent** on the value of **mitochondrial membrane potential**

Mitochondria... Cont.

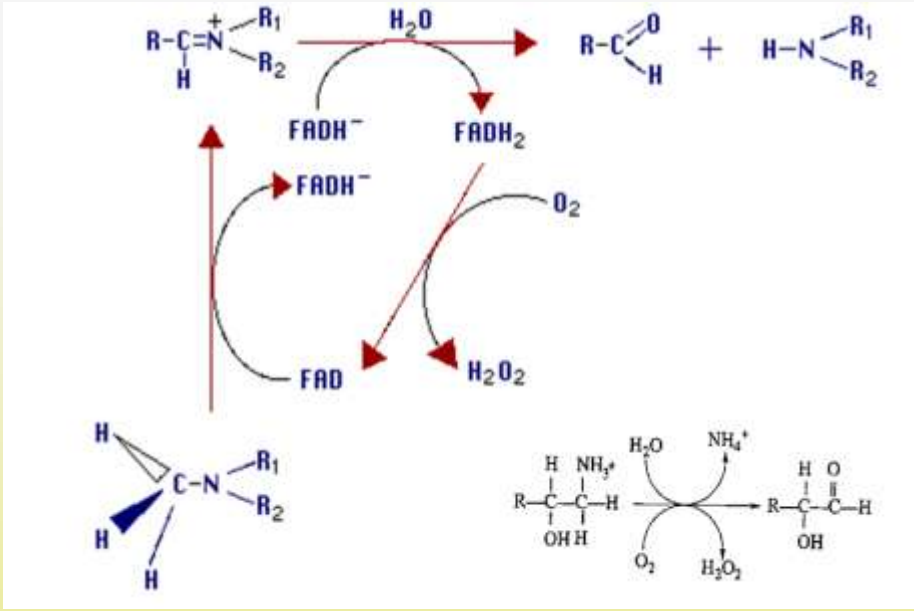
- **Inhibition of neuronal respiration leads to a significant increase in ROS** in mitochondria
- **Overproduction of ROS** in mitochondria **leads to** imbalance & induce oxidative stress & **neurodegeneration**
- This effect can be **reduced by mitochondrial un-couplers (how?)**
- Significant neuroprotection by mild **uncoupling with UCP2 in cerebral stroke**
- **Mutations in mitochondrial complexes I–IV leads to** activation of ROS production & **neuronal cell death**

Monoamine Oxidase



- **Flavoenzymes**
- Mitochondrially located (**outer membrane**)
- Monoamine oxidase A & B (**MAO A & B**); *~70% identical*
- Their role in **oxidative catabolism of important amine neurotransmitters (serotonin, dopamine, & epinephrine)**

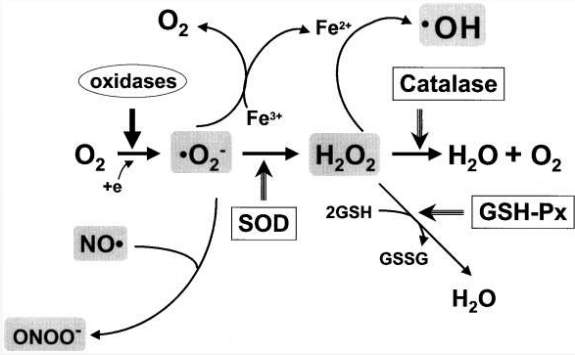
- Expressed in **neurons (MAO-A)** & **glial cells (MAO A & B)**
- MAO breaks down monoamines using **FAD** & results in the production of **aldehydes. The FAD-FADH2 cycle generates hydrogen peroxide**

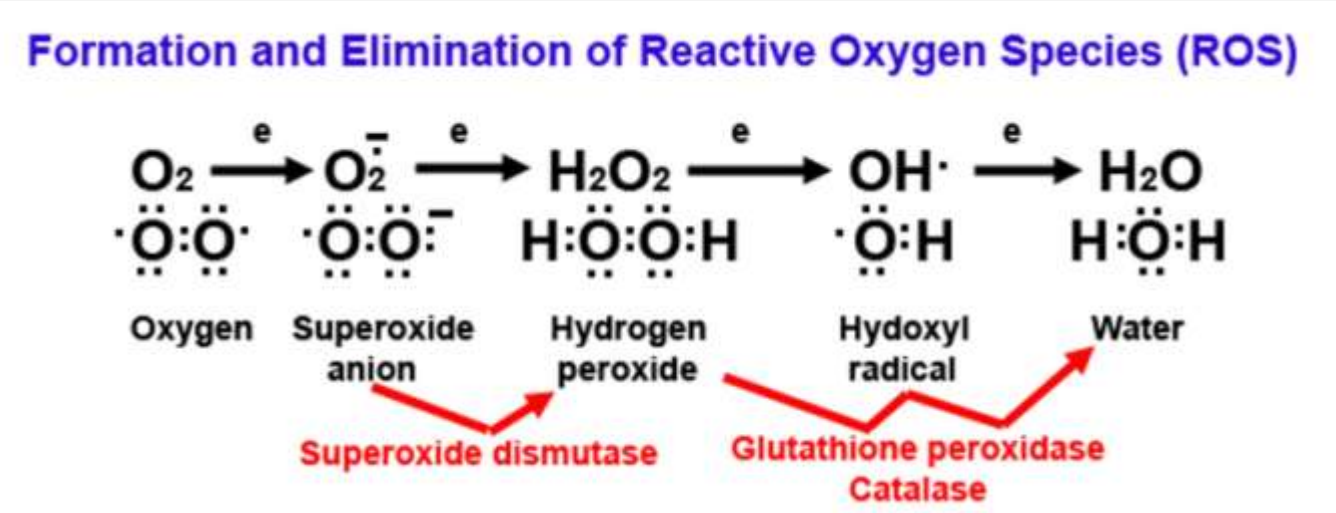


THE ANTIOXIDANT SYSTEM - ENZYMES

Superoxide Dismutases

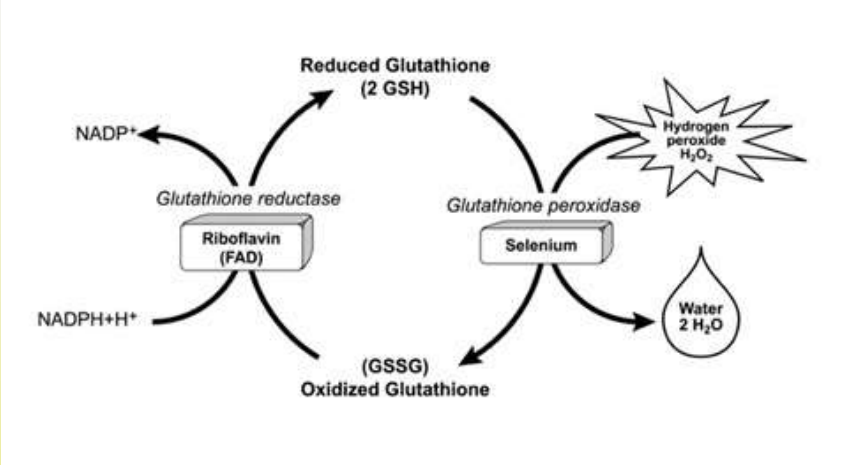
- Play a crucial role in **scavenging O•⁻²**
- **Specialized in** eliminating **superoxide** anion radicals
- Three distinct **isoforms**:
  - **Copper-zinc** superoxide dismutase (Cu/Zn SOD)
  - **Manganese** superoxide dismutase (Mn SOD)
  - **Extracellular** superoxide dismutase (EC SOD)





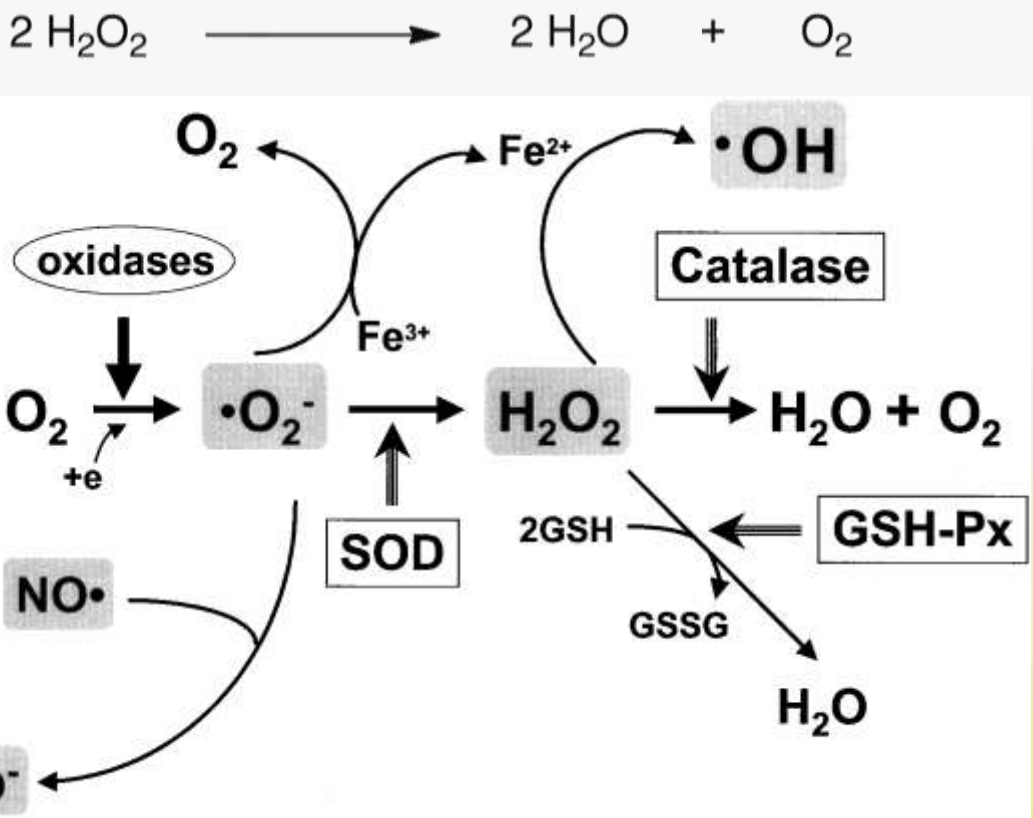
Glutathione Peroxidases

- A family of **multiple isozymes**
- Catalyze the **reduction of H2O2 to water** using reduced **glutathione (GSH) as an electron donor**
- ***(H2O2 + 2GSH → GS-SG + 2H2O)***
- In mammalian tissues, there are **four major selenium-dependent glutathione peroxidases**
- **GPX1 is known to localize primarily in glial cells**, in which GPX activity is **10-fold** higher than in neurons



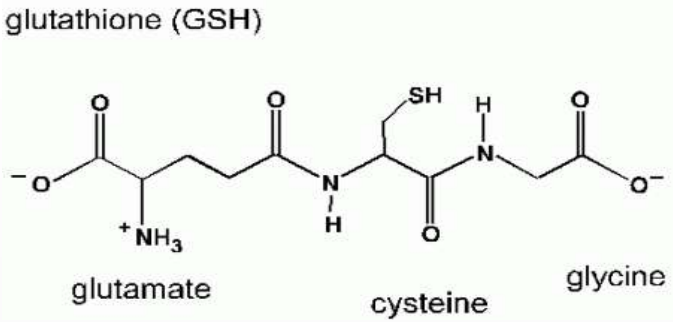
Catalase

- Catalase is a **ferriheme**-containing enzyme
- Converts **hydrogen peroxide to water**
- It is localized in **peroxisomes**, cytoplasm & mitochondria

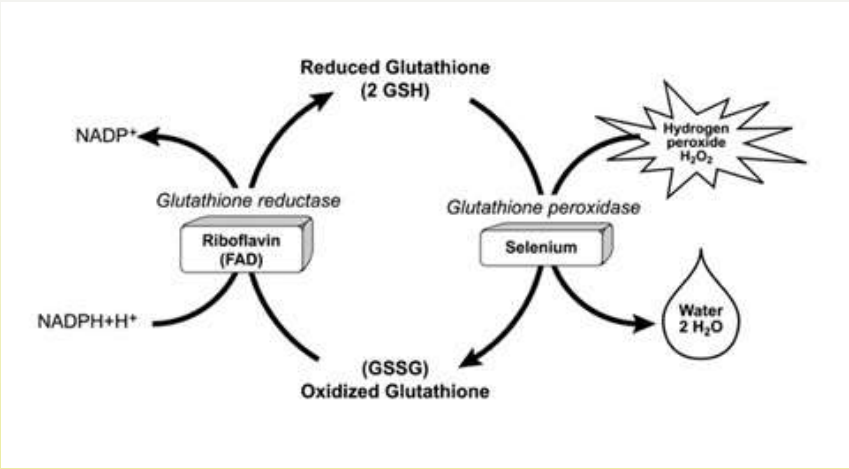


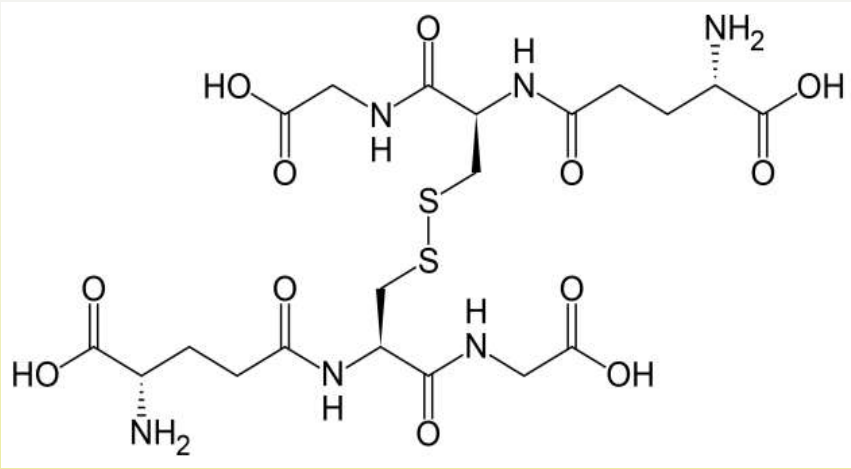
THE ANTIOXIDANT SYSTEM - *NON-ENZYMATIC ANTIOXIDANTS*

GSH

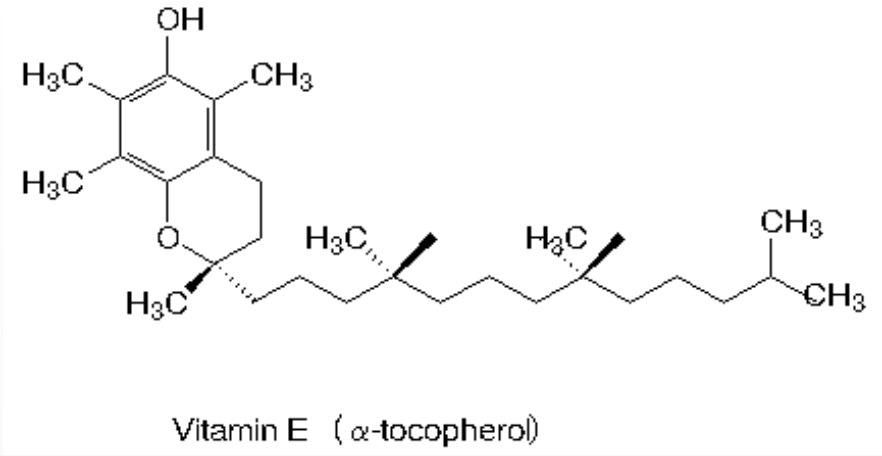


- **The main antioxidant in CNS**
- The most abundant small molecule, non-protein thiol in cells
- Consists of a **tripeptide**
- Reduced GSH can **non-enzymatically act directly with free radicals**, notably superoxide radicals, hydroxyl radicals, nitric oxide, & carbon radicals for their removal
- GSH peroxidase & GSH reductase can act **enzymatically** to remove H2O2 & maintain GSH in a reduced state



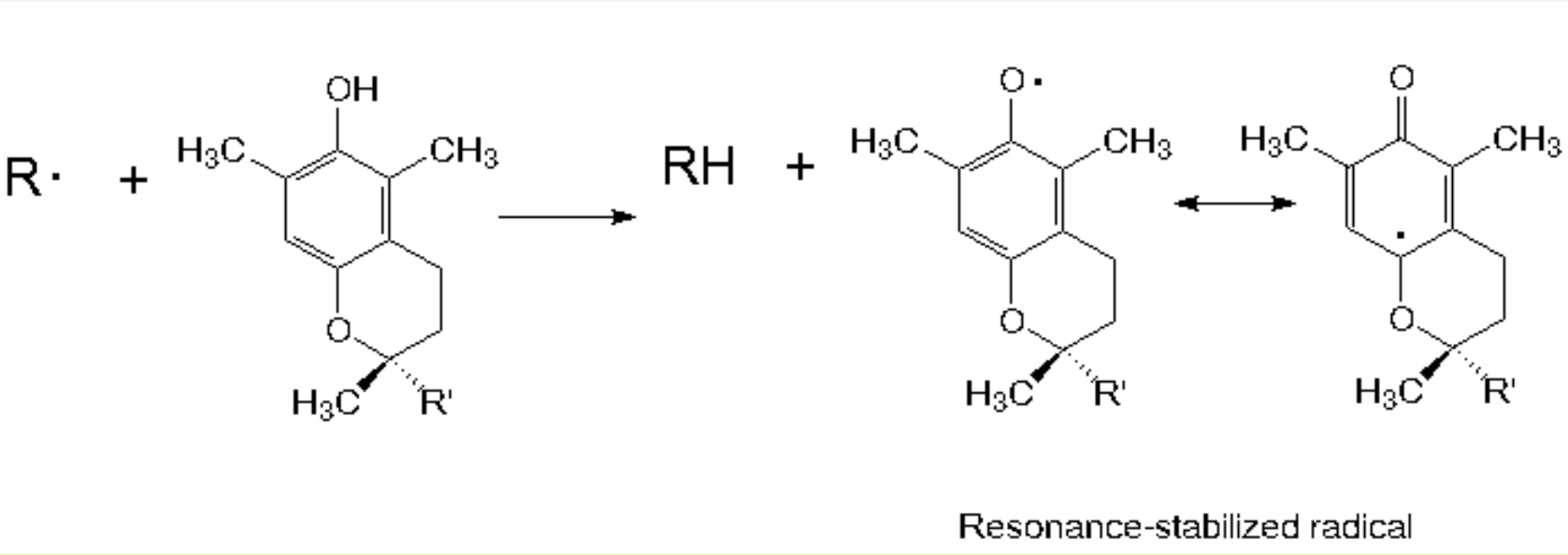


Vitamin E



Vitamin E (α-tocopherol)

- A **lipid soluble** molecule with **antioxidant function** (mainly)
- It appears to neutralize the effect of peroxide & prevent lipid peroxidation in membranes

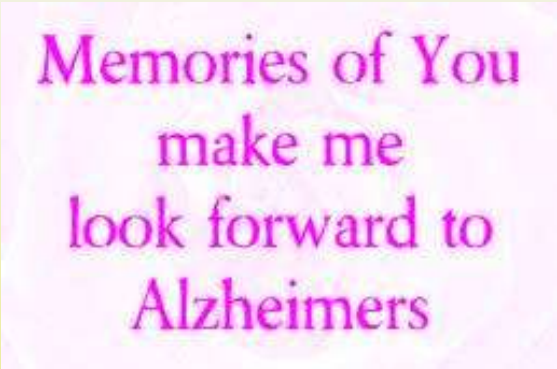


Resonance-stabilized radical

OXIDATIVE STRESS OCCURS IN NEURODEGENERATIVE DISEASES

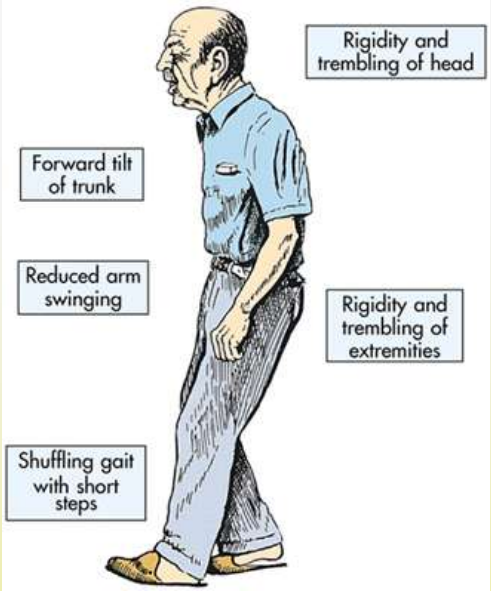
Alzheimer's disease

- The **most common** neurodegenerative disease, affecting approximately 16 million people worldwide
- Characterized by **progressive neuronal loss** associated with **aggregation** of protein as **extracellular amyloid (βA) plaques**, & **intracellular tau tangles**
- AD brains also show **evidence of ROS mediated-injury**;
  - Increase in levels of **malondyaldehyde & 4-hydroxynonenal** in brain & cerebrospinal fluid
  - **Protein carbonyl moieties** are increased in the frontal & parietal cortices, & hippocampus with **sparing of the cerebellum**
  - Increase in **hydroxylated guanosine**



Parkinson's disease

- The **second most common**
- Characterized by progressive **loss of dopaminergic neurons** in the substantia nigra, & **aggregation of the protein α-synuclein**
- Concentration of **PUFAs in the substantia nigra is reduced**, while the levels of lipid peroxidation **markers** (malondialdehyde & 4-hydroxynonenal) **are increased**
- **Protein oxidative damage** in the form of protein carbonyls is also **evident**
- Increased levels of **8-hydroxydeoxygua-nosine**



Mechanisms of Oxidative Stress: ROS Production by Mitochondrial Dysfunction

- Mitochondrial pathology is evident in many neurodegenerative diseases including AD & PD
- The spectrum of **mitochondrial dysfunction** is vast;
  - *Respiratory chain dysfunction*
  - *Oxidative stress*
  - *Reduced ATP production*
  - *Calcium dysregulation*
  - *Mitochondrial permeability transition pore opening*
  - *Deregulated mitochondrial clearance (mitophagy)*

ROS Production by Mitochondrial Dysfunction; **PD**

- **A reduction in complex I activity** in the substantia nigra
- The neurotoxin 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine **(MPTP) has been shown to produce parkinsonian symptoms**
- l-methyl-4-phenylpyridinium **(MPP+)**, the active metabolite of MPTP, can **block ETC** (same site as **rotenone**)
- **Rotenone or MPP+ also produces superoxide** anions in sub-mitochondrial particles
- Mild **uncoupling** of mitochondria with **UCP2** overexpression **reduces ROS production** (MPP+, rotenone)
- The identification of a number of **PD-related genes** that are strongly **associated with mitochondrial function (PINK1, DJ-1, & Parkin)**

oxidative stress is a primary event in **PD** pathogenesis

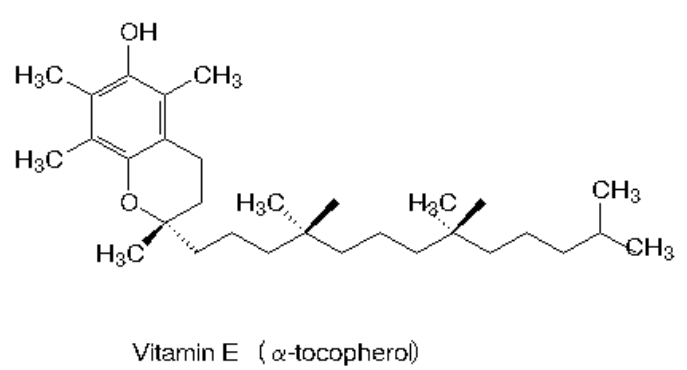
- **Mutations in PINK1** (mitochondrial kinase) **cause a recessive form of PD**
- **PINK1 deficiency** results in **inhibition of complex I**, & rotenone-like **increased** production of **ROS** in mitochondria
- Abnormal **aggregation** of protein **α-synuclein**, **which accumulates in all PD brain**
- **Mutations in α-synuclein gene cause a familial form of autosomal dominant PD**
- Expression of **mutant α-synuclein** in neurons results in **increased ROS** production
- **α-synuclein** binds mitochondria & **induce mitochondrial fragmentation**

ROS Production by Mitochondrial Dysfunction; AD

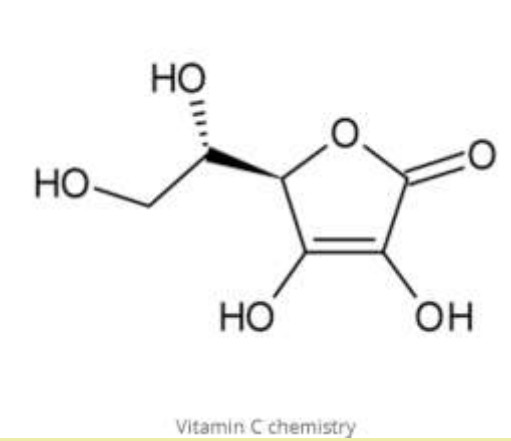
- **A reduction in complex IV activity** in mitochondria from the **hippocampus**
- **Deregulation of calcium homeostasis**;
  - **βA causes** increased cytoplasmic calcium levels & **mitochondrial calcium overload**, resulting in increase in **ROS production & opening of the PTP**
- **βA directly interact with cyclophilin D** (a PTP component) forming a complex in the mitochondria that has **reduced threshold for opening**
- **Fragmented mitochondria** are seen in AD **hippocampus**

Use of Antioxidant Therapy in Neurodegenerative Disease

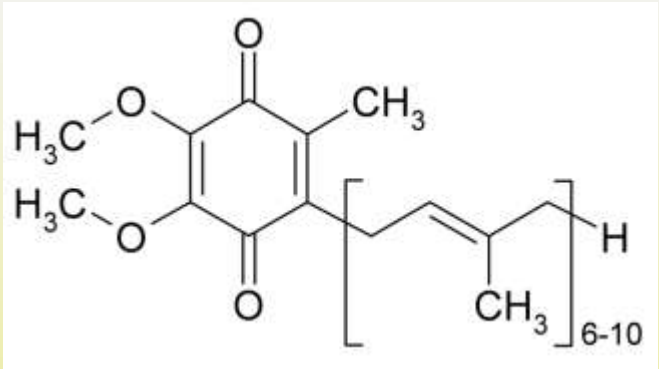
- The rationale for the use of antioxidants as therapies is clear
- The benefits of antioxidants in animal & cell models of disease was promising
  - *Vitamin E*
  - *Vitamin C*
  - *Coenzyme Q*



Vitamin E (α-tocopherol)



Vitamin C chemistry



Promising !

- **Vitamin E** supplementation in **AD** mouse model resulted in **improved cognition & reduced βA deposition**
- **AD;** Daily injections of **vitamin C** in mouse model significantly **reduced memory deficits**
- **PD; Coenzyme Q** has been shown to have multiple **protective effects** within the mitochondria
- **PD;** CoQ **protects** MPTP-treated mice from **dopaminergic neuronal loss & also attenuated α-synuclein aggregation**

Promising but!

- There has been **no proven benefit for the use of vitamin E &/or vitamin C in either AD or PD** from large randomised controlled clinical trials

- **Vitamin E**, **CoQ**, & **glutathione** clinical trials in PD concluded that there were **only minor treatment benefits in the CoQ trials** that may have been due to **improvement in the respiratory chain deficit rather than a direct antioxidant action**

- **None of the trials have shown significant benefit to warrant recommendation for use in the clinical setting!!!!**

Promising but!

- All **animal models are limited** in recreating the human disease
  - **long-time frame**
  - **Gradual accumulation** of age-related changes
- Antioxidants must be administered at an **early stage** where the process influences pathogenesis most
- The **bioavailability** of reducing molecules in the human brain in the doses used in animal models
- The **effective targeting** of such molecules to the mitochondria in human brain
- **Several different producers** of oxidative stress in each disease (need to be targeted separately but simultaneously)