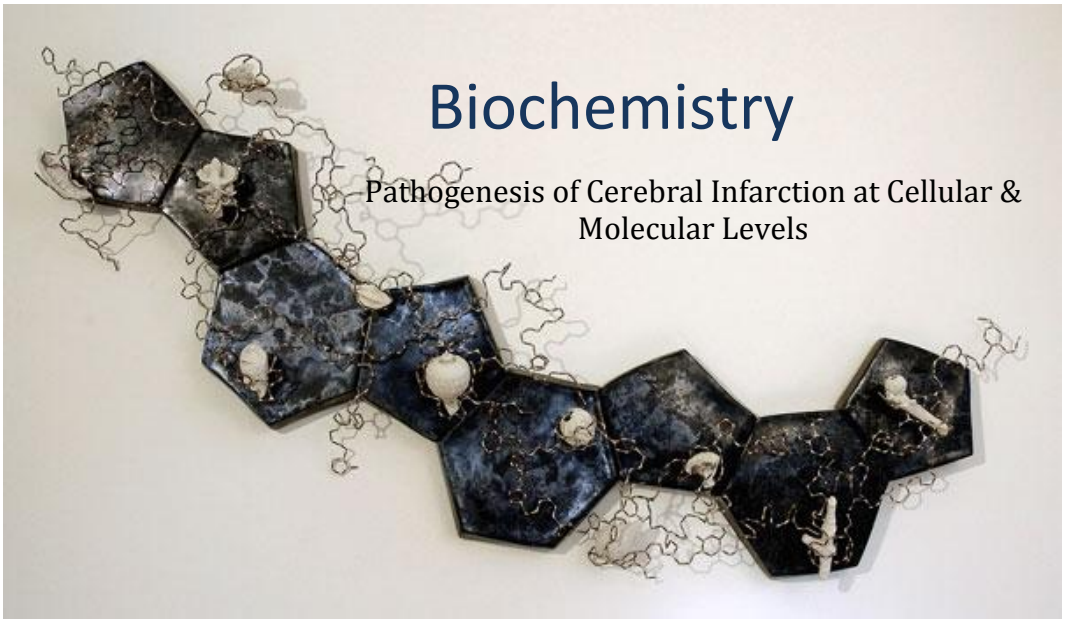

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The **Cell Death Mechanisms** Implicated In The Pathogenesis Of Ischemic Brain

Note: 20 sec of loss of blood supply to the brain is enough to start the ischemic effects .

Stroke = Cerebral infarction.

- **Necrosis:**
  - is commonly observed **early** after **severe** ischemic insults
- **Apoptosis:** "programmed cell death"
  - occurs with more **mild** insults and with **longer** survival periods "necrosis may happen in apoptosis"

- The mechanism of cell death involves calcium-induced **calpain-mediated proteolysis** of brain tissue *remember: calpain = calcium induced pain

- **Substrates for *calpain* include:**
  - ❑ Cytoskeletal proteins
  - ❑ Membrane proteins
  - ❑ Regulatory and signaling proteins

Increase intracellular ca+ caused from :

-outside " ↑ca+ influx "

-inside "storage withen the endoplasmic reticulum.

- **Biochemical Responses to Ischemic Brain Injury** :
  A. **Oxidative stress**
  B. **Metabolic stress**
  C. **Neurochemical response**

When does the Oxidative stress occur ?

It occurs as a result of imbalance between oxidant & antioxidant reaction.

i.e.

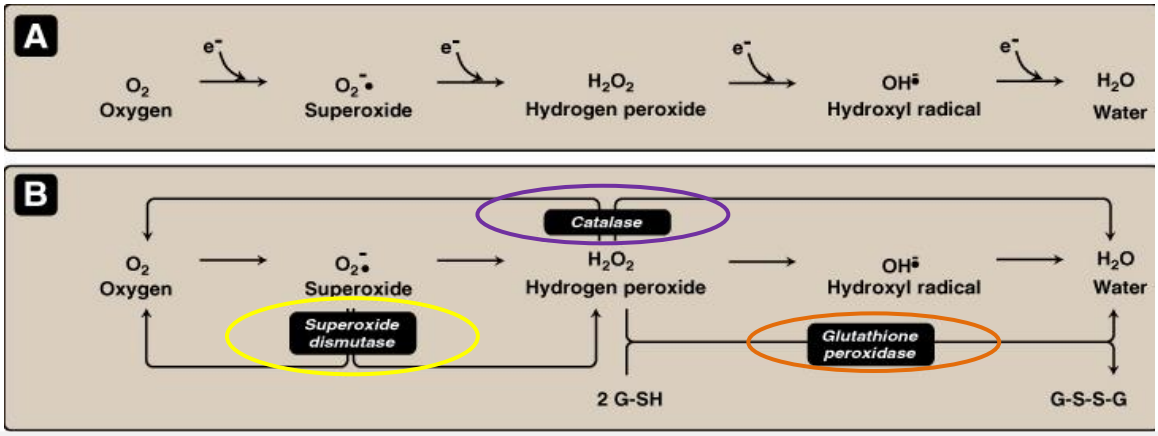
1. ↑oxidant , ↓antioxidant. Or 2. Normal oxidant ↓antioxidant.

Or

3. ↑oxidant , Normal antioxidant.

i. Oxidative stress

- **A condition in which cells are subjected to excessive levels of Reactive Species (Oxygen or nitrative species) & they are unable to counterbalance their deleterious effects with antioxidants.**
- **It has been implicated in the ageing process & in many diseases (e.g., atherosclerosis, cancer, neurodegenerative diseases, stroke, diabetes)**



-Superoxide dismutase converts $O_2^{-\bullet}$ to H_2O_2 which is less toxic , because it's not a pure free radical and can be eradicated easily.

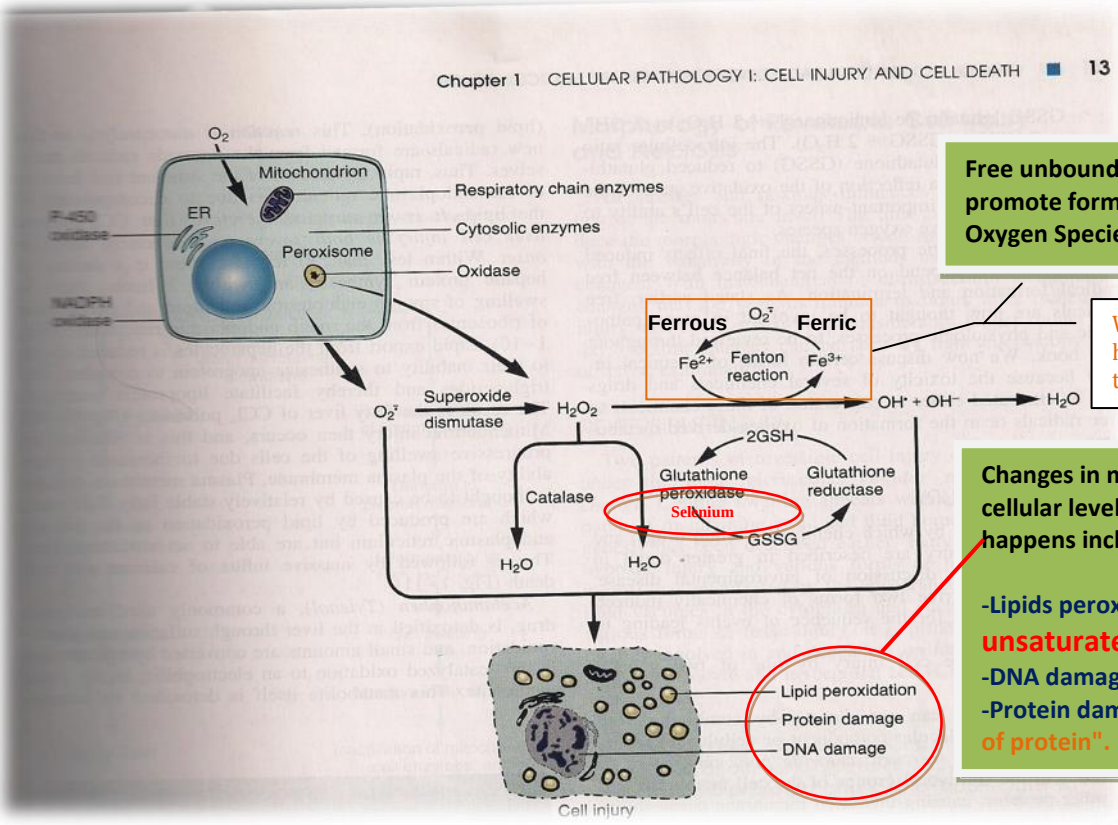
-Superoxide dismutase is antioxidants even if it does not eradicate $O_2^{-\bullet}$ completely.

-This process has to be gradual or it will cause a burst , and it takes place in mitochondria in respiratory chain.

-Hydroxyl radicals is formed in presence of H_2O_2 and Fe^{2+}.

-Catalase converts H_2O_2 to water and O_2, so the body get rid of it.

Glutathione peroxidase converts H_2O_2 to water.



Free unbound iron can also promote formation of Reactive Oxygen Species

Which will converts hydrogenperoxide to hydrogen radical

Changes in molecular and cellular level when cell death happens include :

-Lipids peroxidation of **unsaturated** fatty acids.
-DNA damage.
-Protein damage "denaturation of protein".

CONT'D :
-Glutathione system includes: (glutathione, NADP H_2, reductase, peroxidase & selenium)
- Selenium : chemical element is a cofactor for Glutathione peroxidase.
- Free Ferrous (Fe^{2+}): it will react with H_2O_2 to form $OH\cdot$ and OH^- .

- In the reduced form 2(G-SH) are oxidized by glutathione peroxidase to form 1(GS-SG).
-glutathione peroxidase is an enzyme that requires selenium to do its function.
-The oxidized form (GS-SG) can be reduced to (G-SH) by the enzyme glutathione reductase with the help of an electron donor (NADPH + H +).

The Role of Reactive Oxygen Species (ROS) & Reactive Nitrative Species (RNS) in Normal Brain Physiology

Remember :
-Any kinase does phosphorylation to the protine .

- They are mainly generated by microglia & astrocytes
- They modulate synaptic transmission & non-synaptic communication between neurons & glia
- During periods of increased neuronal activity, ROS & RNS diffuse to the myelin sheath of oligodendrocytes activating Protein kinase C (PKC) → posttranslational modification of myelin basic protein (MBP) by phosphoryylation
- They regulate neuronal signaling in both central & peripheral nervous systems
- They are required for essential processes as learning & memory formation

The brain and Oxidative stress

The brain is highly susceptible to ROS-induced damage because of:

- High concentrations of peroxidisable lipids
- Low levels of protective antioxidants
- High oxygen consumption
- High levels of iron (acts as pro-oxidants under pathological conditions)
- The occurrence of reactions involving dopamine & Glutamate oxidase in the brain "because they utilize the oxygen"

Why this reaction is important in the brain ?
-To form the neurotransmitters.

peroxidisable lipid : is a lipid containing unsaturated fatty acid that can be peroxidized if exposed to ROS.

Molecular & Vascular effects of ROS in ischemic stroke

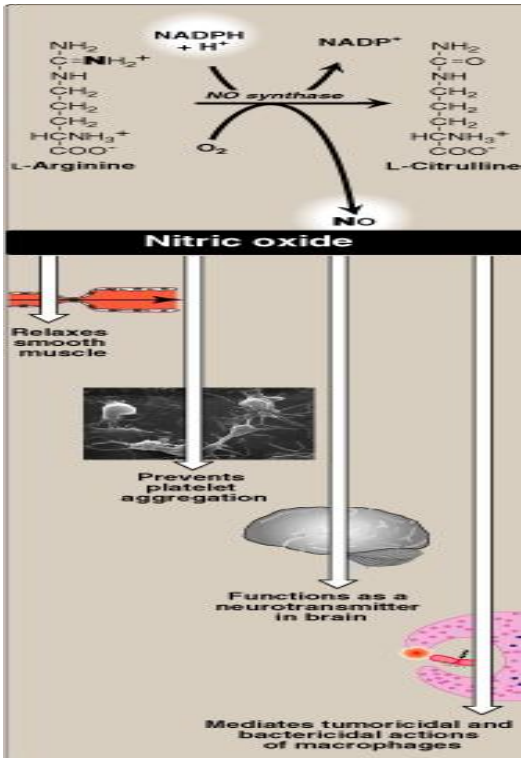
Remember : **Reactive oxygen species** are mainly *divided* into non-free radicals e.g hydrogen peroxide and free radicals e.g Superoxide

- **Molecular effects:**
  - **DNA damage**
  - **Lipid peroxidation of *unsaturated fatty acids***
  - **Protein denaturation**
  - **Inactivation of enzymes**
  - **Cell signaling effects (e.g., release of Ca^{2+} from intracellular stores)**
  - **Cytoskeletal damage**
  - **Chemotaxis**

- **Vascular effects:**
  - **Altered vascular tone and cerebral blood flow**
  - **Increased platelet aggregability "ROS → thrombus formation"**
  - **Increased endothelial cell permeability**

The role of NO in the pathophysiology of cerebral ischemia

- **Ischemia→ abnormal NO production**
- **This may be both beneficial and detrimental, depending upon when and where NO is released**
- **NO produced by endothelial NOS (eNOS) → improving vascular dilation and perfusion (i.e. beneficial).**
- **In contrast, NO production by neuronal NOS (nNOS) or by the inducible form of NOS (iNOS) has been reported to has detrimental effects on outcome (harmful) .**
- **Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes**



Explanation:

Nitric Oxide (NO) :
Synthesis:
Enzyme: nitric oxide (NO) synthase
Precursor: L-Arginine
Effects:
Relaxes vascular smooth muscle
Prevents platelet aggregation
Bactricidal & Tumoricidal effects
Neurotransmitter in brain

Nitric Oxide (NO) :

Beneficial
eNOS produced by endothelial NOS
Function : dilation and perfusion

Detrimental
iNOS, nNOS
produced by neuronal nNOS or by inucible form of iNOS
iNOS is associated with inflammation

ii. Metabolic stress

○ Biochemical changes in The brain during ischemia

Ischemia → interruption or severe reduction of blood flow, O_2 & nutrients in cerebral arteries → energy depletion (depletion of ATP & creatine phosphate)

1. Inhibition of ATP-dependent ion pumps
   - Membranes depolarization
   - Perturbance of transmembrane ion gradients(membranes damage)

(anaerobic glycolysis)↑ **Lactic acid** in neurons → acidosis → promotes the pro-oxidant effect → ↑ the rate of conversion of O_2^- to H_2O_2 or to hydroxyperoxyl radical

- **Ca^{2+} Influx** (translocation from extracellular to intracellular spaces) → activation of cellular proteases (Calpains) & lipases → breakdown of cerebral tissue

"**Ca^{2+} Influx** -> disturbance in many enzymes e.g. proteases, lipases, ATPases , phospholipases ….. "

- **Na^+ influx**
- **K^+ efflux**
- K^+-induced **release of excitatory** amino acids

Sources &consequences of increased calcium in cell injury .

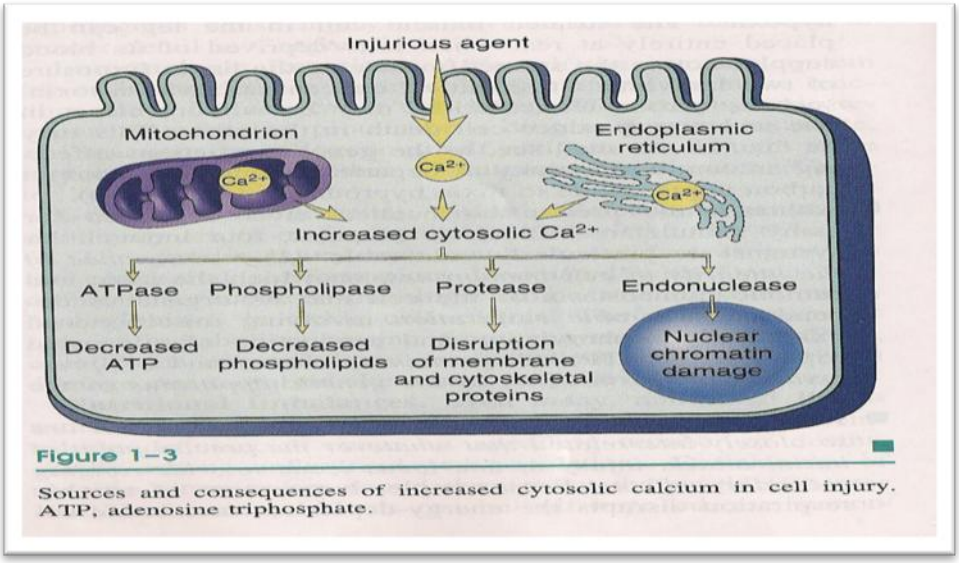


Figure 1–3

Sources and consequences of increased cytosolic calcium in cell injury. ATP, adenosine triphosphate.

Ca^{2+} will come from 3 sources :
-Injurious agent
- Mitochondria
- Endoplasmic reticulum

Then, what will happen ?
-Decreased ATP by stimulation ATPase.
- increase breakdown of phospholipids results in fomrmation of Injurious products (e.g Free fatty acids) when they accumulate they cause membrane damage (mitochondrial membrane, plasma membrane , lysosomal membranes)
- Disruption of membrane and cytoskeletal proteins
- Nuclear chromatin damage

iii. Neurochemical response

The neurochemical response to cerebral ischemia

❑ **Following cerebral ischemia extracellular levels of various neurotransmitters are increased e.g., Glutamate , Glycine ,GABA , Dopamine**

Biochemical basis of pharmacological intervention

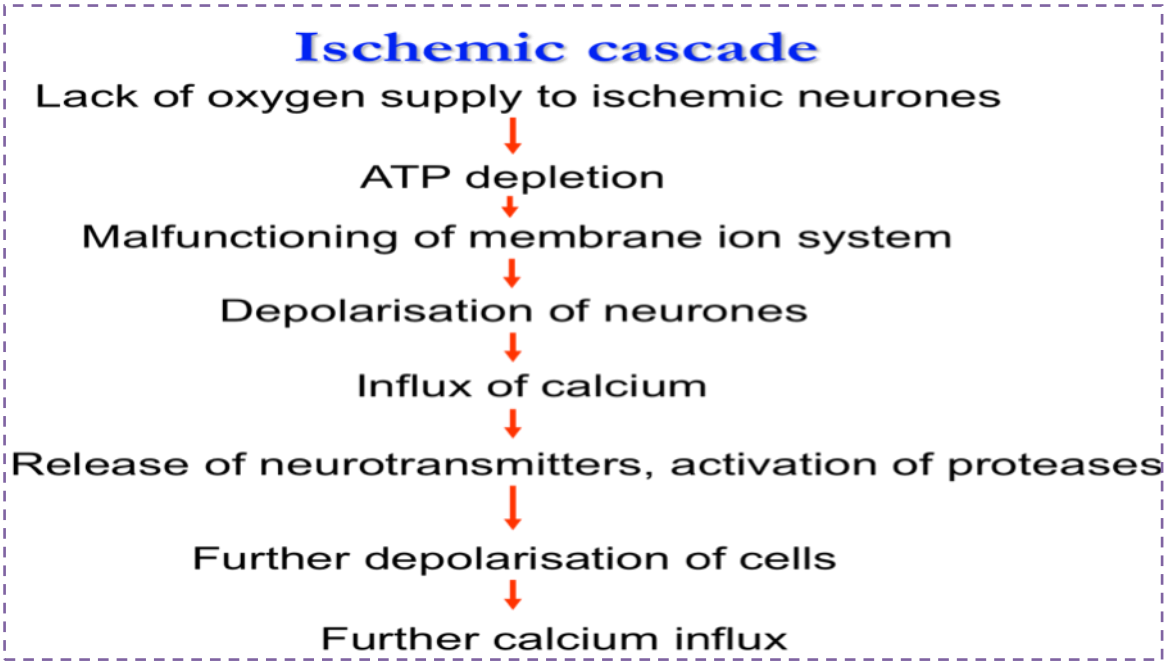
Examples of Potential Biochemical Intervention in Cerebral Ischemia

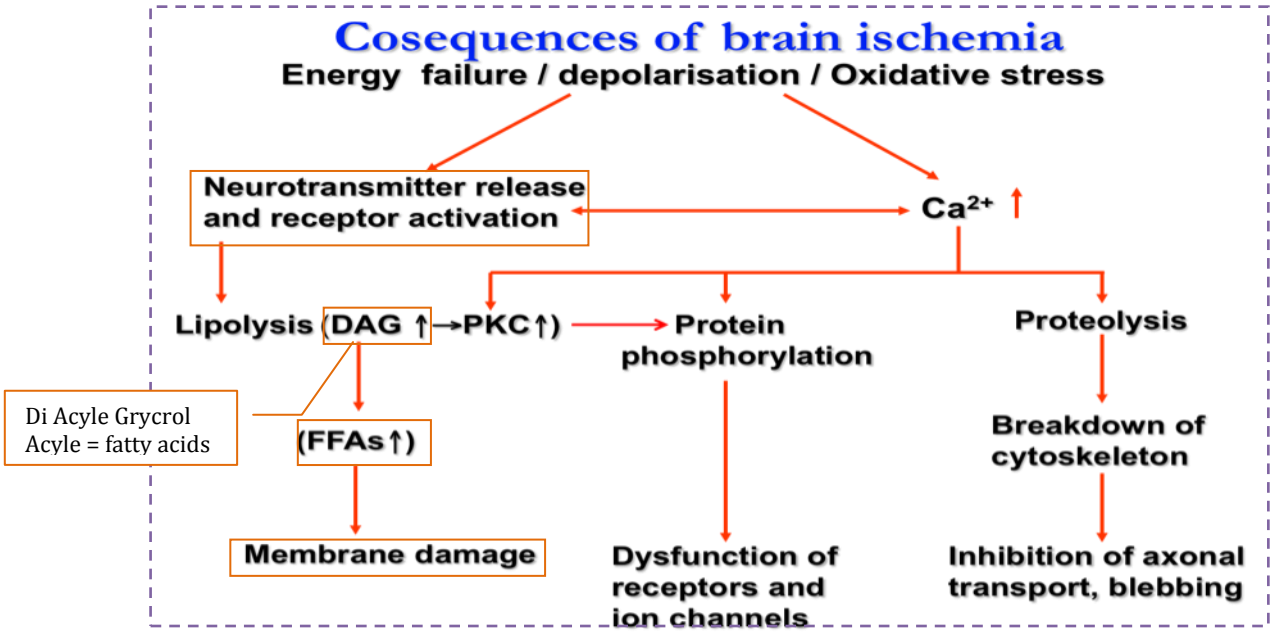
- **Inhibitors of glutamate release**
- **Ca^{2+} channel blockers**
- **Nitric oxide synthase inhibitors & free radical inhibition**
- **Calpain inhibitors**

Severe cerebral ischemic insults lead to a complex cascade of biochemical and molecular events, including:

1. **Cell death**
2. **Oxidative stress**
3. **Metabolic stress and neurochemical changes**

To summarize:





Review Questions

1-Which one of the followings is an antioxidant ?

A-Superoxide
B-Glutathione reductase
C-hydrogen peroxide
D-Calpains

2-Which one of the following occurs as a result of Inhibition of ATP-dependent ion pumps

A-Increased Na+ influx leading to inactivation of lipases & proteases causing mambrain damage
B- K+-induced release of excitatory amino acids
C-No perturbance of transmembrane ion gradients
D-non above

3-which one of the following result from Molecular effects of ROS in ischemic stroke

A- Lipid peroxidation of saturated fatty acids
B- Altered vascular tone and cerebral blood flow
C- Protein denaturation
D-non above

4- which of the following is a beneficial type of nitric oxides

A- endothelial NOS (eNOS)
B-the inducible form of NOS (iNOS)
C- neuronal NOS

5- what's the main event in ischemia?

A - cl influx
B - ca influx
C - Na efflux
D - K influx

1-b
2-b
3-c
4-A
5- B

Done By

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