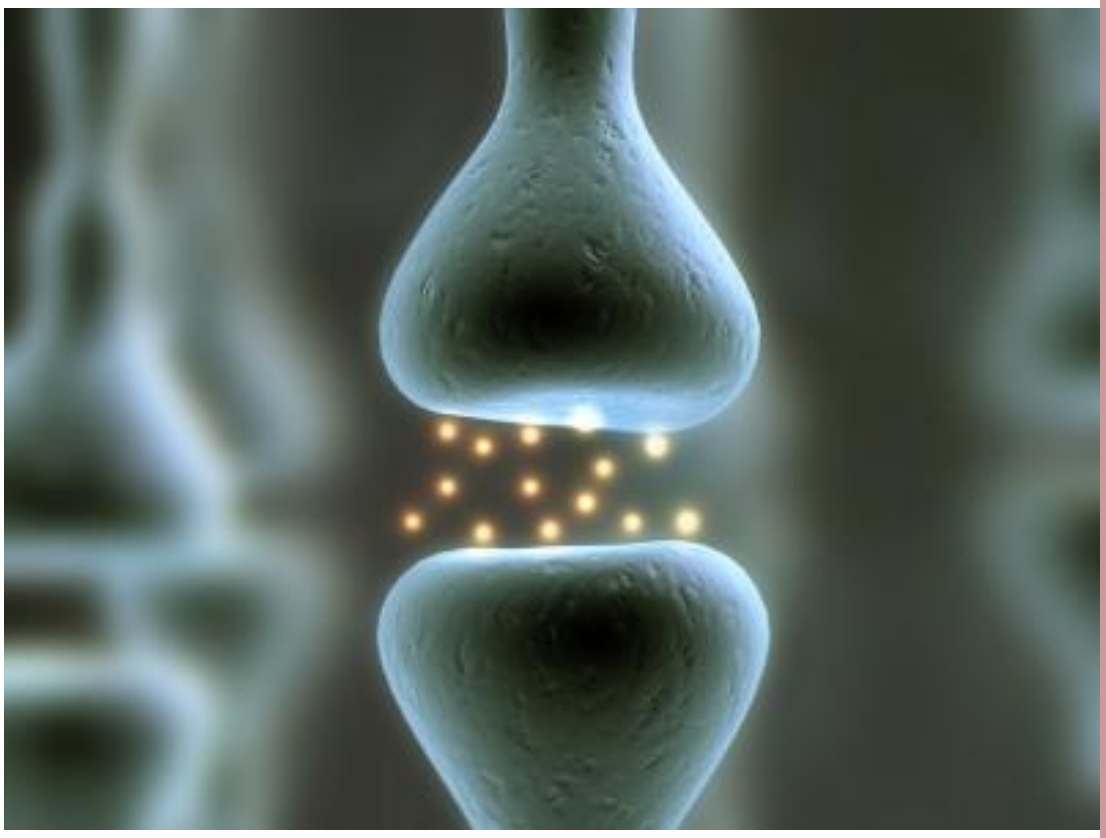
Biochemistry of the CNS



3rd lecture:

Pathogenesis of Cerebral Ischemia

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❖ The cell death mechanisms implicated in the pathogenesis of ischemic brain injury :

1) **Necrosis:** is commonly observed early after severe ischemic insults
   With almost complete blockade of the blood vessels that supply the affected cell

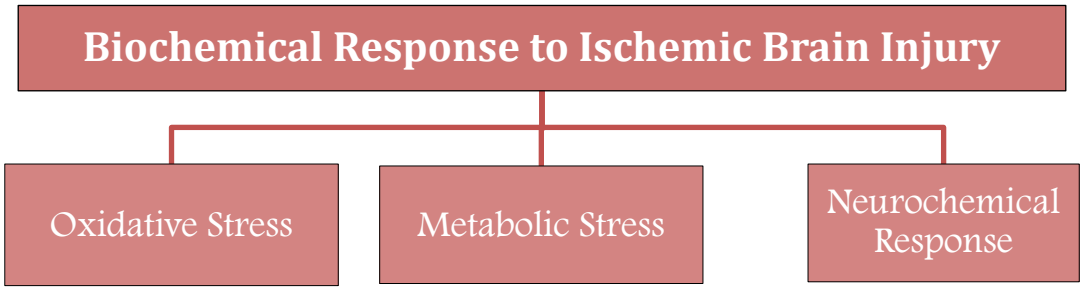
2) **Apoptosis:** occurs with more mild insults and with longer survival periods
   With narrowing of the blood vessels that supply the affected cell

- The mechanism of cell death involves calcium-induced calpain-mediated proteolysis of brain tissue (calcium is increased in cerebral ischemia → activates calpain → tissue death)

- Substrates for calpain include:
  1. Cytoskeletal proteins
  2. Membrane proteins
  3. Regulatory and signaling proteins

Proteolysis :
destruction & degradation of proteins

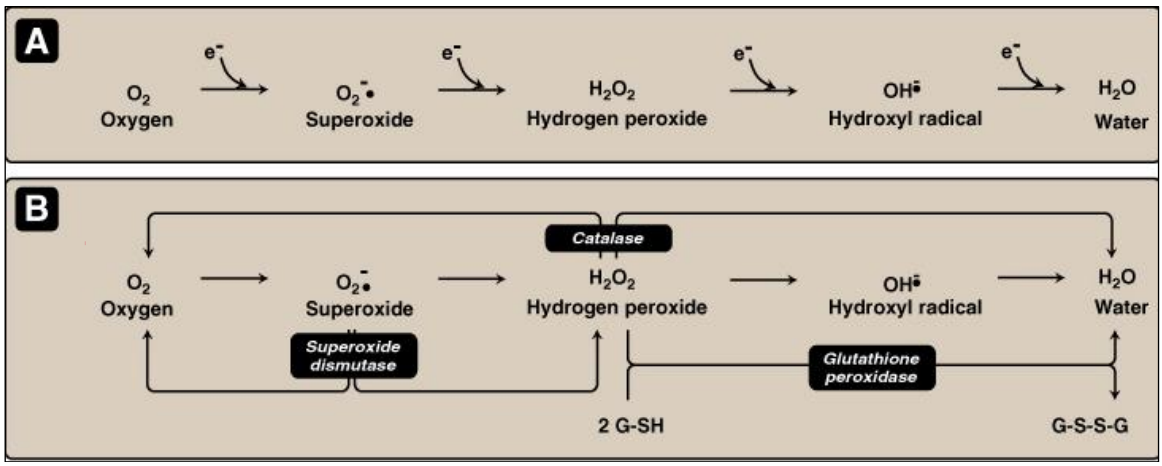
" As any enzyme needs substrate , proteolytic enzymes needs proteins as substrates "



♣ 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)



- When one step reduction happens it causes explosion, therefore, it occurs in steps.
- Through this process, some free radicals and/or reactive oxygen species are formed but they are under control as long as we have normal level of antioxidants with normal function.
- These previous molecules must be cleaved in the body by antioxidants in order not to do harm. So the body is not under oxidative stress normally.
- There are some vitamins that are considered to be part of the antioxidant group such as vitamin C and carotenes.

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

DNA → mutation (first to be affected)

Lipids → peroxidation
if they were unsaturated

Protein → denaturation

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

- 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 phosphorylation

  Protein kinase C (PKC) → activated by reactive oxygen species and phosphorylates other proteins → posttranslational modification of myelin basic protein (MBP).

- 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

❖ Molecular & Vascular effects of ROS in ischemic stroke :

- **Molecular effects:**
  - DNA damage
  - Lipid peroxidation of unsaturated fatty acids
  - Protein denaturation
  - Inactivation of enzymes
  - Cell signaling effects (e.g., release of Ca2+ from intracellular stores)
  - Cytoskeletal damage
  - Chemotaxis

- **Vascular effects:**
  - Altered vascular tone and cerebral blood flow
  - Increased platelet aggregability
  - Increased endothelial cell permeability

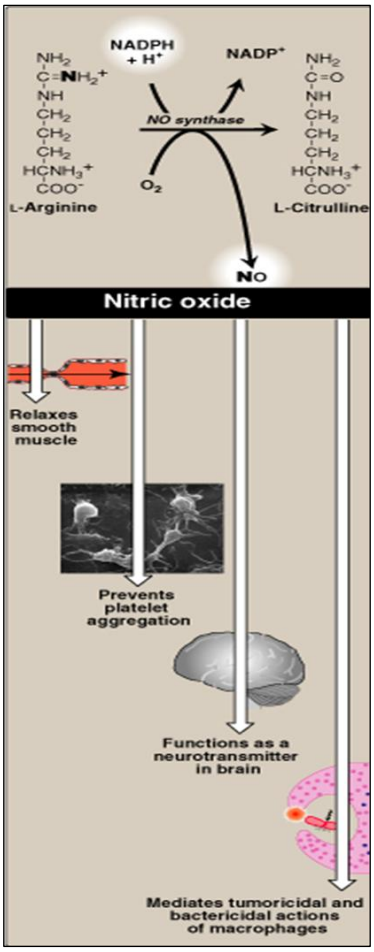
Vascular changes lead to edema of the brain

❖ 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

NOS *(nitric oxide synthase)* family of enzymes, can be classified according to their location, *and* availability *(inducible or not):*

- 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 have detrimental effects on outcome.
- Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes.



Only the effects of NO are required

- Relaxes smooth muscles
- Prevents platelet aggregation
- Functions as a neurotransmitter in brain
- Mediate tumoricidal & bactericidal actions of macrophages

♣ Metabolic Stress :

❖ Biochemical changes in The brain during ischemia :

Ischemia → interruption or severe reduction of blood flow, O2 & nutrients in cerebral arteries → energy depletion (depletion of ATP & creatine phosphate)

- Inhibition of ATP-dependent ion pumps
  - Membranes depolarization
  - Perturbance of transmembrane ion gradients

↑ **Lactic acid** in neurons → acidosis → promotes the pro-oxidant effect → ↑ the rate of conversion of O2- to H2O2 or to hydroxyperoxyl radical

- **Ca^{2+} Influx** (translocation from extracellular to intracellular spaces) → activation of cellular proteases (Calpains) & lipases → breakdown of cerebral tissue
  - **Na^{+} influx**
  - **K^{+} efflux**
- K^{+}-induced **release of excitatory** amino acids

Sources & consequences of increased cytosolic Calcium in cell injury :

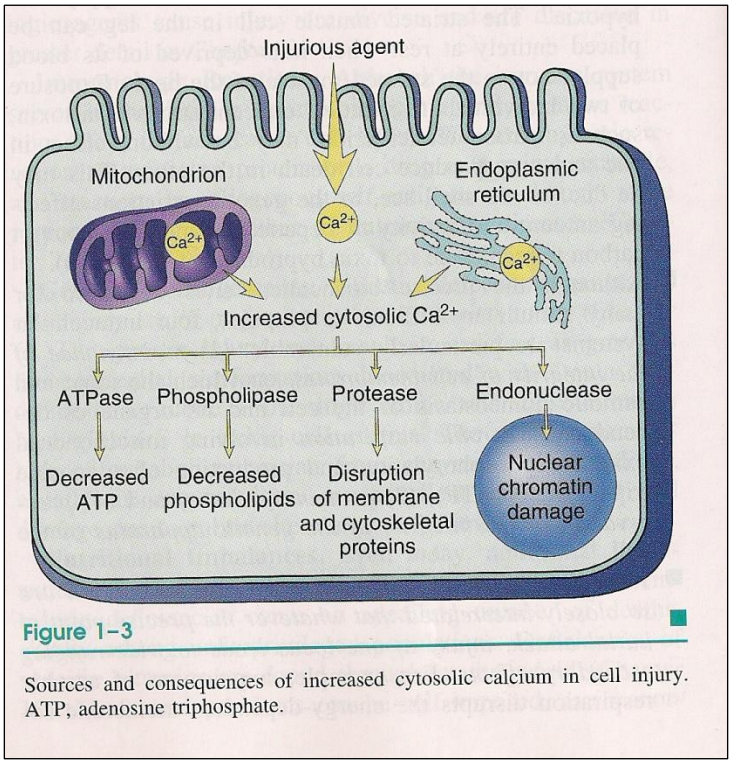


Figure 1–3

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

❖ **Sources :**

- Injurious agent
- Mitochondria
- Endoplasmic reticulum

❖ **Consequences :**

- Decreased ATP
- Decreased phospholipids
- Disruption of membrane and cytoskeletal proteins
- Nuclear chromatin damage

♣ Neurochemical Response :

❖ The neurochemical response to cerebral ischemia :

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

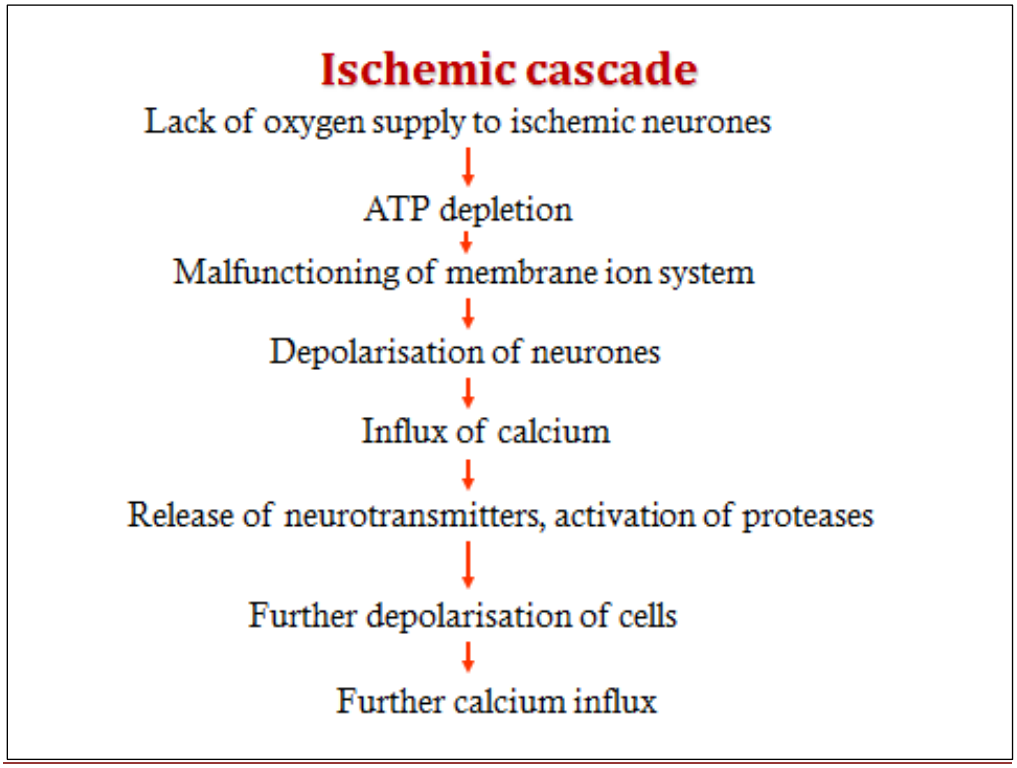
♣ Biochemical Basis of Pharmacological Intervention :

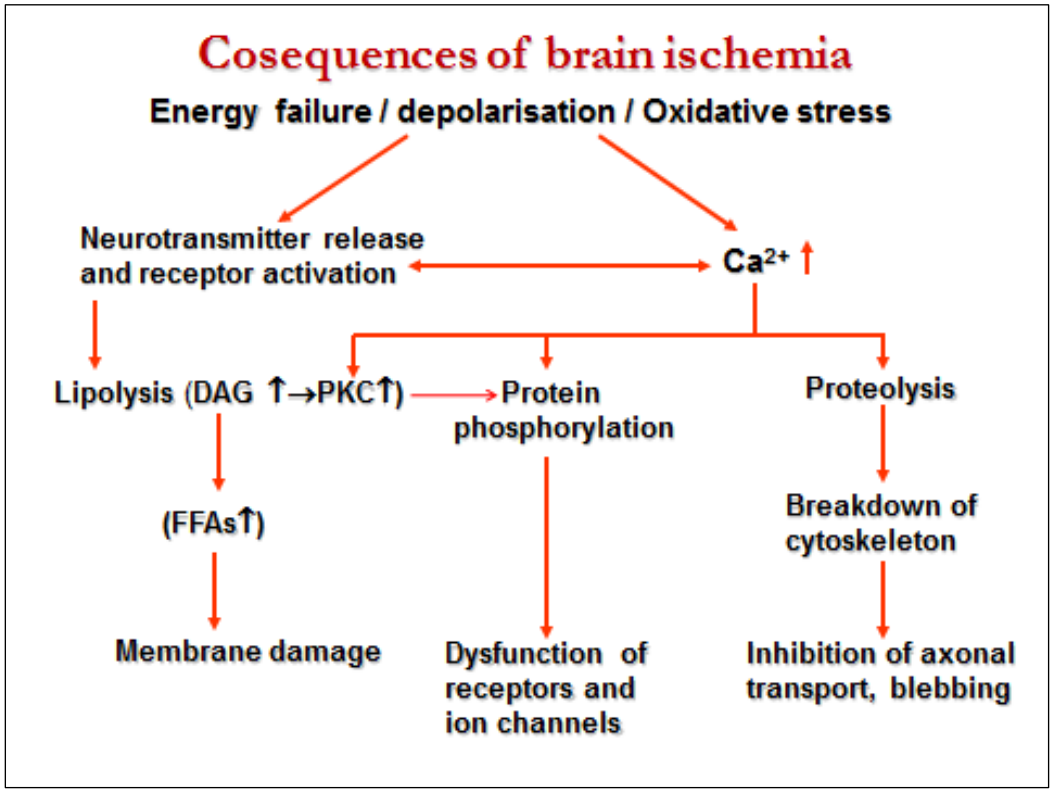
❖ Examples of Potential Biochemical Intervention in Cerebral Ischemia :

- Inhibitors of glutamate release
- Ca2+ channel blockers
- Nitric oxide synthase inhibitors & free radical inhibition
- Calpain inhibitors

Summary

- Severe cerebral ischemic insults lead to a complex cascade of biochemical and molecular events, including:
  - Cell death
  - Oxidative stress
  - Metabolic stress and neurochemical changes


Ischemic cascade
Lack of oxygen supply to ischemic neurones
ATP depletion
Malfunctioning of membrane ion system
Depolarisation of neurones
Influx of calcium
Release of neurotransmitters, activation of proteases
Further depolarisation of cells
Further calcium influx


Cosequences of brain ischemia
Energy failure / depolarisation / Oxidative stress
Neurotransmitter release and receptor activation
Ca2+ ↑
Lipolysis (DAG ↑→PKC↑)
Protein phosphorylation
Proteolysis
(FFAs↑)
Breakdown of cytoskeleton
Membrane damage
Dysfunction of receptors and ion channels
Inhibition of axonal transport, blebbing