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**Pathogenesis of Cerebral  
Infarction  
at  
Cellular & Molecular Levels**

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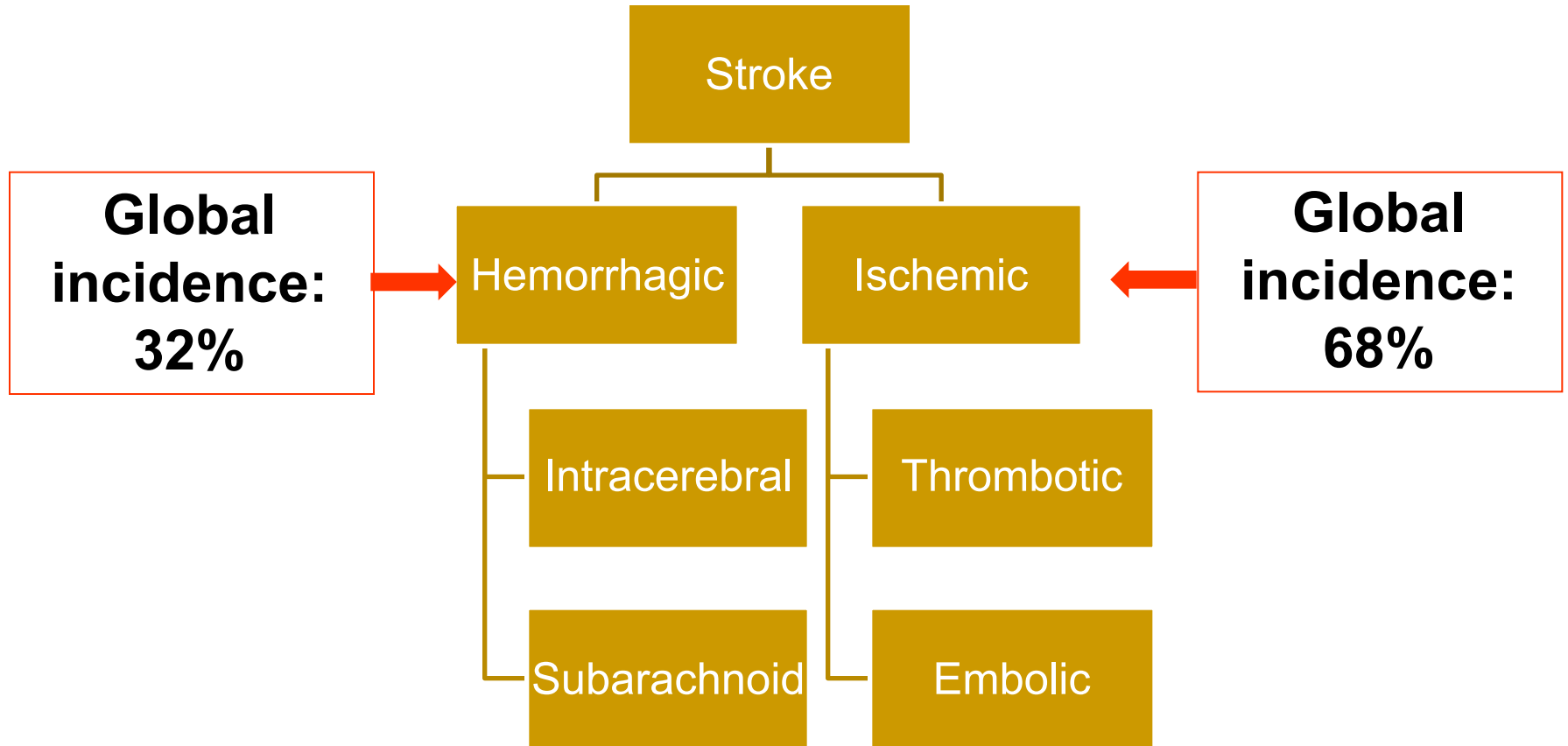
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# Objectives:

**By the end of this lecture, the students should be able to:**

- Identify the possible **cell death mechanisms** implicated in the pathogenesis of ischemic brain injury
  - Acquire the knowledge of the important role played by **oxidative stress and free radicals** in the pathogenesis of cerebral infarction
  - Understand the various factors involved in **ischemia-induced metabolic stress**
  - Identify the Neurochemical changes involved in cerebral ischemia
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# Cerebral Ischemia (Strokes) subtypes



# Risk factors of strokes

- There are a number of risk factors for stroke:
  - Some increase the risk of one type of stroke (hemorrhagic or ischemic).
  - Some increase the risk of both types.
  - Occasionally, strokes occur in people who have no risk factors.

*Continued .....*



# Risk factors of strokes

## Ischemic stroke risk factors

Age older than 40 years  
Heart disease  
High blood pressure  
Smoking  
Diabetes  
High blood cholesterol levels  
Illegal drug use  
Recent childbirth  
Previous history of transient ischemic attack  
Inactive lifestyle and lack of exercise  
Obesity  
Current or past history of blood clots  
Family history of cardiac disease and/or stroke

## Hemorrhagic stroke risk factors

High blood pressure  
Smoking  
Illegal drug use (especially cocaine and "crystal meth")  
Use of warfarin or other blood thinning medicines

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

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# Cell death mechanisms in cerebral ischemia: Necrosis and Apoptosis

- **Necrosis** is commonly observed **early** after **severe** ischemic insults
- **Apoptosis** occurs with more **mild** insults and with **longer** survival periods
- The mechanism of cell death involves calcium-induced **calpain-mediated proteolysis** of brain tissue
- **Substrates for calpain include:**  
Cytoskeletal proteins, Membrane proteins and Regulatory and signaling proteins

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# **Biochemical Responses to Ischemic Brain Injury**

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**Oxidative stress**

**Metabolic stress**

**Neurochemical response**



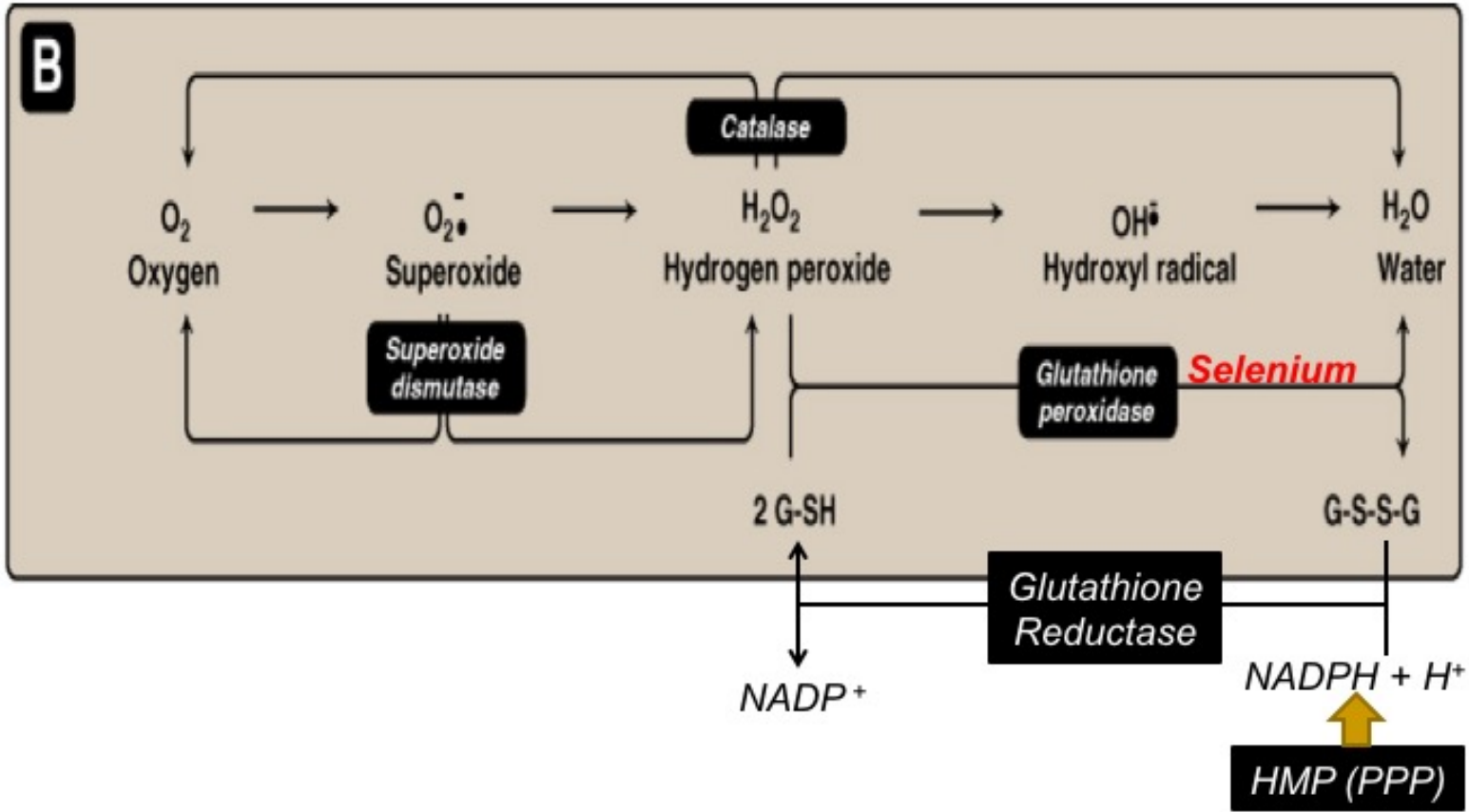
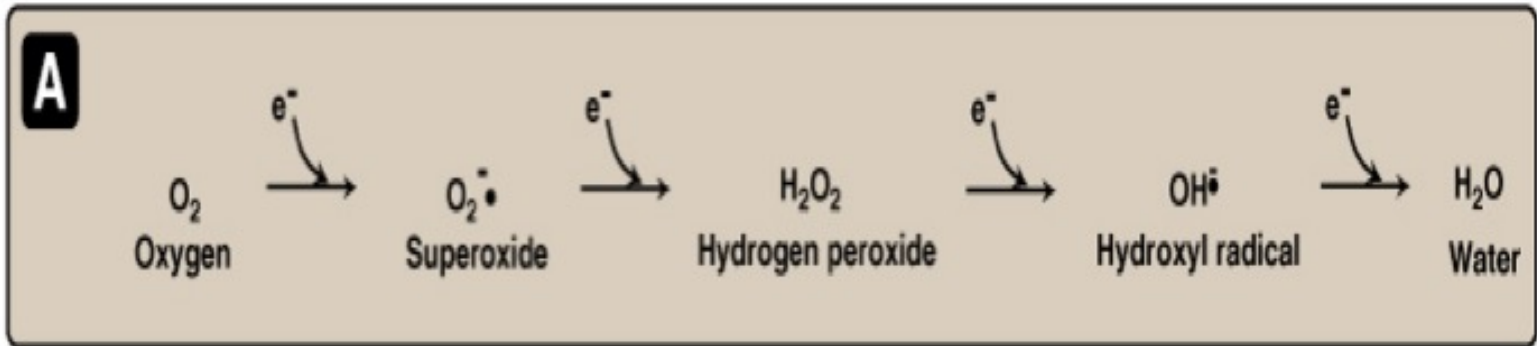


# **Oxidative stress**



# The Role of Reactive Oxygen Species (ROS) & Reactive Nitrate 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
- They regulate neuronal signaling in both central & peripheral nervous systems
- They are required for essential processes as learning & memory formation

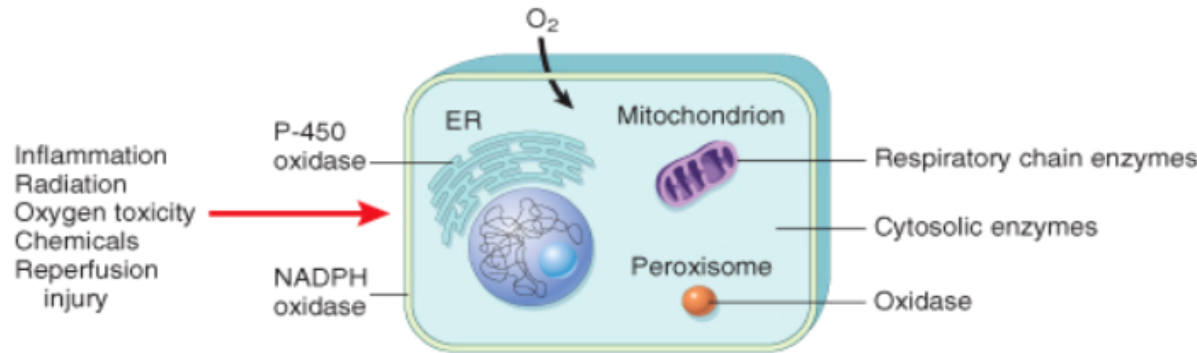


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# Oxidative stress

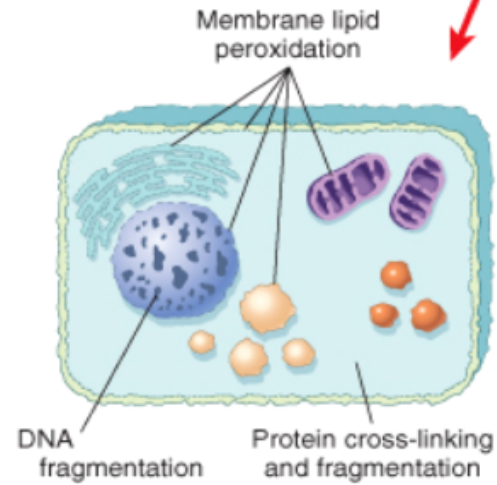
- A condition in which cells are subjected to excessive levels of Reactive oxidizing species (ROS or RNS) & they are unable to counterbalance their deleterious effects with antioxidants.
  - It has been implicated in the ageing process & in many diseases (e.g., atherosclerosis, cancer, neurodegenerative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



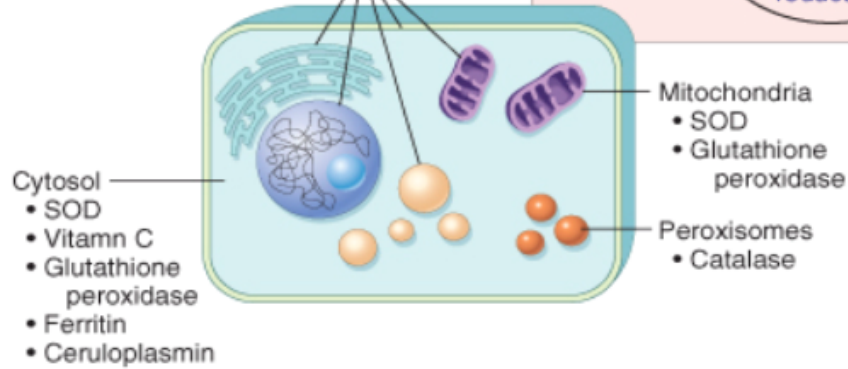
**Reactive oxygen species:**  
 $O_2^{\cdot-}$ ,  $H_2O_2$ ,  $OH^{\cdot}$

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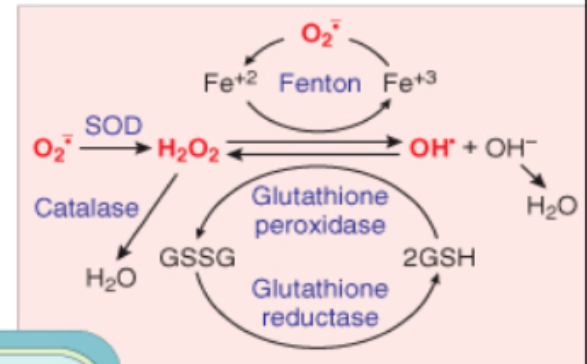


**B. CELL INJURY BY FREE RADICALS**

All membranes  
• Vitamins E and A  
•  $\beta$ -carotene



**C. NEUTRALIZATION OF FREE RADICALS – NO CELL INJURY**



# 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  $\text{Ca}^{2+}$  from intracellular stores)
- ❑ Cytoskeletal damage
- ❑ Chemotaxis

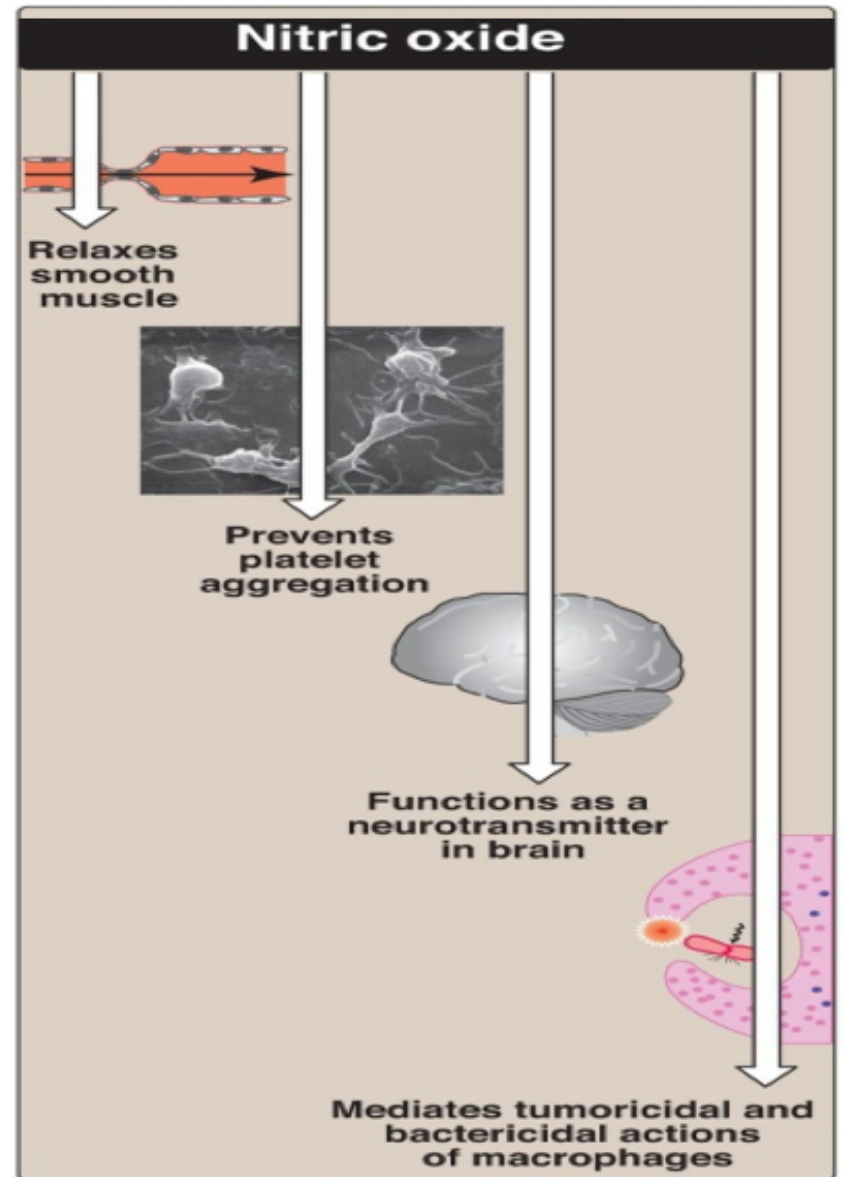
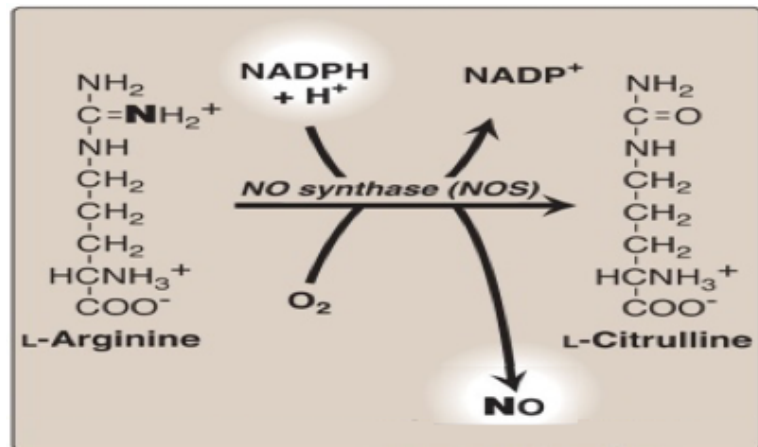
## ■ Vascular effects:

- ❑ Altered vascular tone and cerebral blood flow
  - ❑ Increased platelet aggregability
  - ❑ Increased endothelial cell permeability
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# 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 **detrimental** (harmful) effects.
- Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes







# **Metabolic stress**



# Biochemical changes in The brain during ischemia

Ischemia → interruption or severe reduction of blood flow, O<sub>2</sub> & nutrients in cerebral arteries → **energy depletion** (depletion of ATP & creatine phosphate)

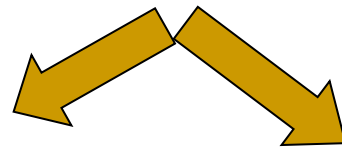
- Inhibition of ATP-dependent ion pumps
  - Membranes depolarization
  - Perturbance of transmembrane ion gradients



• **Ca<sup>2+</sup> Influx** (translocation from extracellular to intracellular spaces) → activation of cellular proteases (Calpains) & lipases → breakdown of cerebral tissue

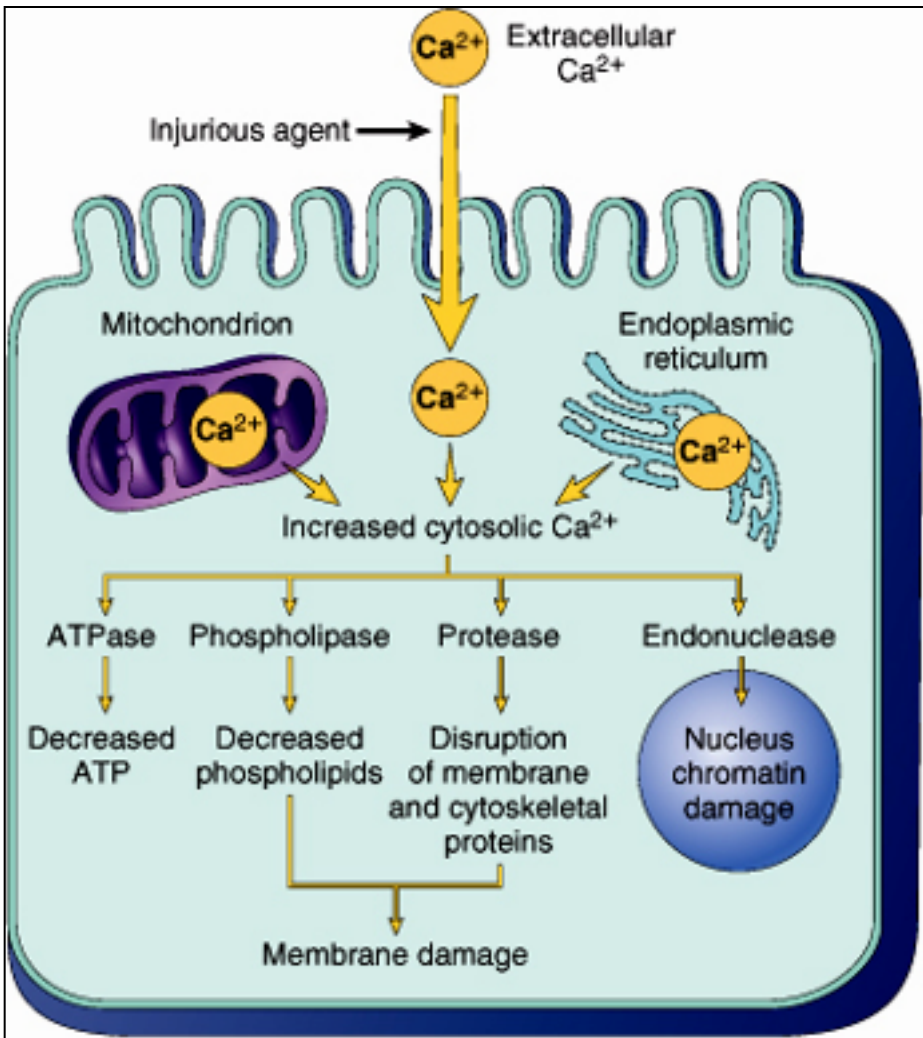
- **Na<sup>+</sup> influx**
- **K<sup>+</sup> efflux**

• K<sup>+</sup>-induced **release of excitatory** amino acids



↑ **Lactic acid** in neurons → acidosis → promotes the pro-oxidant effect → ↑ the rate of conversion of O<sub>2</sub><sup>·-</sup> to H<sub>2</sub>O<sub>2</sub> or to hydroxyperoxyl radical

# Sources & consequences of increased cytosolic Calcium in cell injury





# **Neurochemical response**

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# The neurochemical response to cerebral ischemia

- Following cerebral ischemia, extracellular levels of various neurotransmitters are increased e.g.,
    - Glutamate
    - Glycine
    - GABA
    - Dopamine
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# The Blood tests in patients with brain ischemia or hemorrhage

- Complete blood count, including hemoglobin, hematocrit, white blood cell count, and platelet count
- Prothrombin time, international normalized ratio (INR), and activated partial thromboplastin time
- Thrombin time and/or ecarin clotting time if patient is known or suspected to be taking a direct thrombin inhibitor or a direct factor Xa inhibitor
- Blood lipids, including total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides.
- Cardiac enzymes and troponin

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# **Biochemical basis of pharmacological intervention**

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# Examples of Potential Biochemical Intervention in Cerebral Ischemia

- Inhibitors of glutamate release
  - $\text{Ca}^{2+}$  channel blockers
  - Nitric oxide synthase inhibitors & free radical inhibition
  - Calpain inhibitors
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**To Summarize:**



# 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

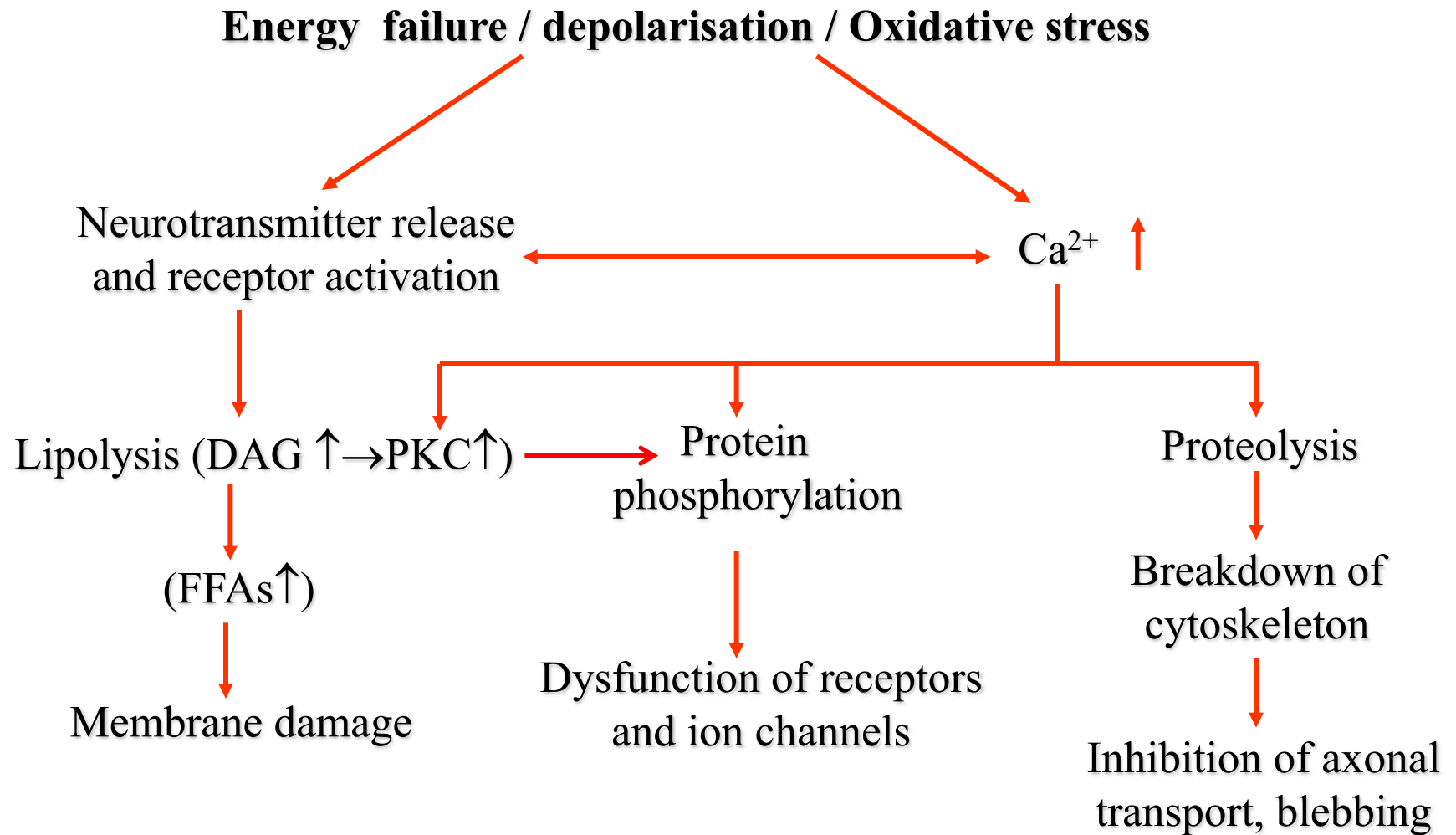


Further depolarisation of cells



Further calcium influx

# Cosequences of brain ischemia



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# Take Home Message

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
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# References

- Lippincott's Illustrated reviews: Biochemistry 6<sup>th</sup> edition, Unit 2, Chapter 13, Pages 145-156.
  - □ Role of Oxidative Stress in Chronic Diseases (Book). □ ([Link](#))
  - The Role of Neurotransmitters in Brain Injury (Book, Page 36). ([Link](#))
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  - Allen and Bayraktutan, Oxidative Stress and its Role in the Pathogenesis of Ischemic Stroke, World Stroke Organization International Journal of Stroke, 2009, 4:461-470