Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

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Objectives:

- 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

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

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
  - Regulatory and signaling proteins

Biochemical Responses to Ischemic Brain Injury

Biochemical Responses to Ischemic Brain Injury

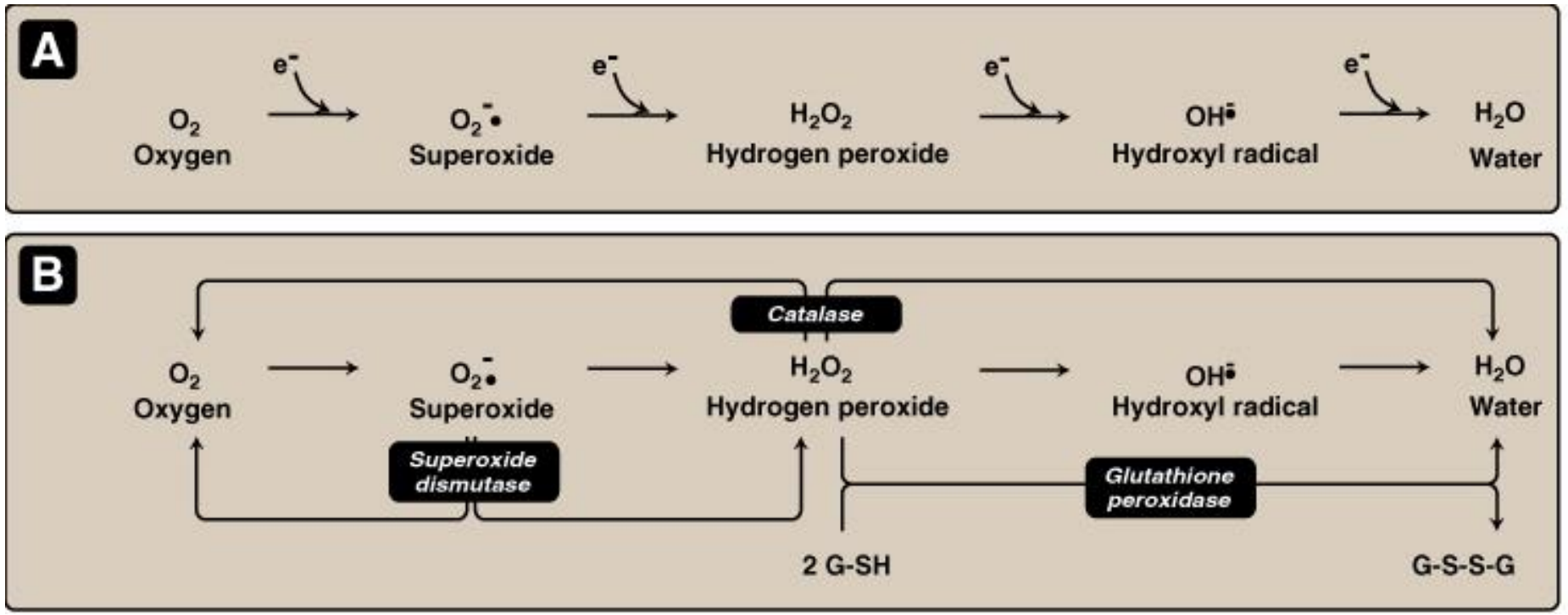
- Oxidative stress
- Metabolic stress
- Neurochemical response

Oxidative stress

Oxidative stress

- A condition in which cells are subjected to excessive levels of Reactive oxidizing 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)


A
e⁻
e⁻
e⁻
e⁻
O2
Oxygen
O2⁻•
Superoxide
H2O2
Hydrogen peroxide
OH•
Hydroxyl radical
H2O
Water
B
Catalase
O2
Oxygen
O2⁻•
Superoxide
H2O2
Hydrogen peroxide
OH•
Hydroxyl radical
H2O
Water
Superoxide dismutase
Glutathione peroxidase
2 G-SH
G-S-S-G

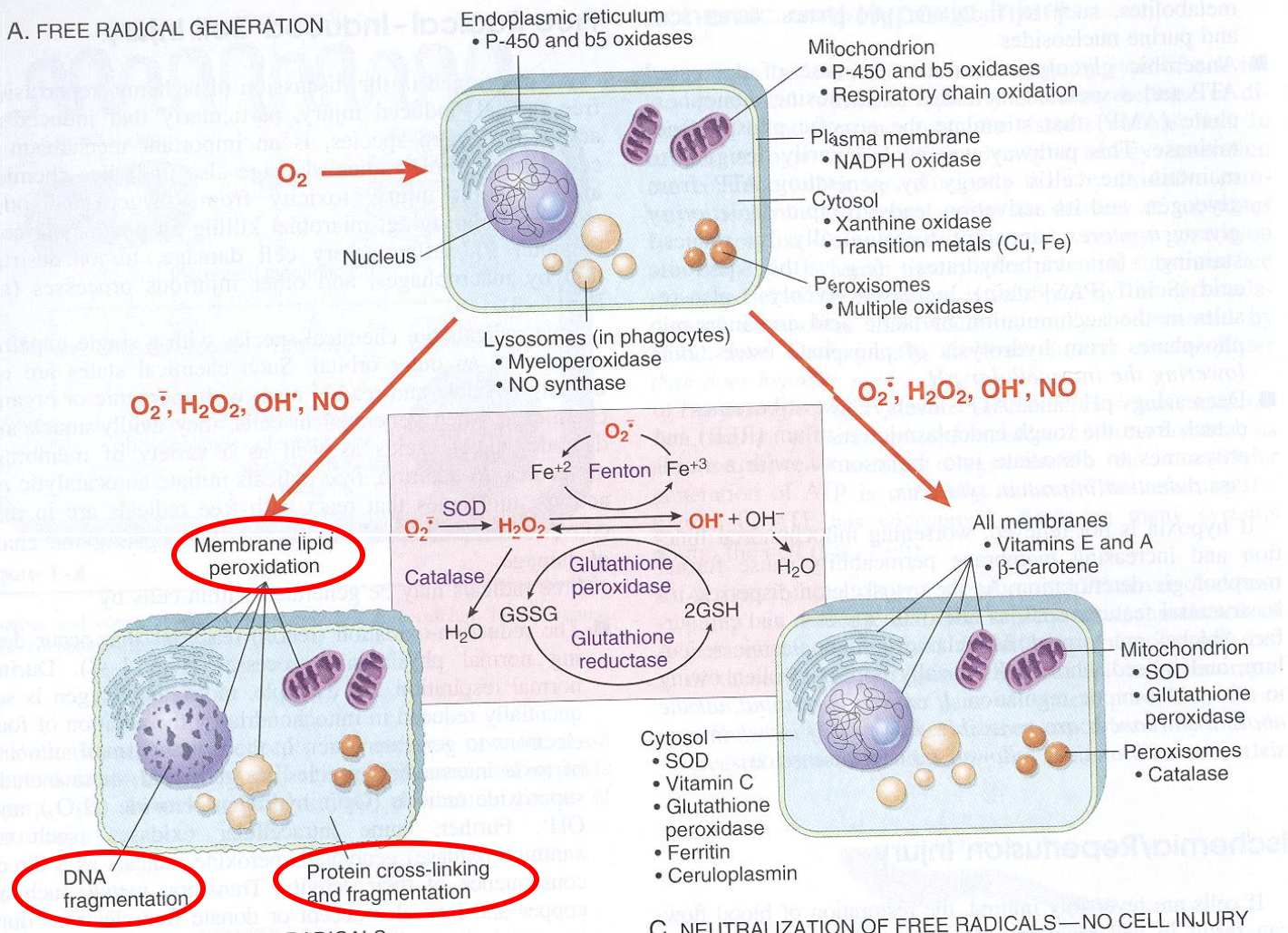

A. FREE RADICAL GENERATION
Endoplasmic reticulum
• P-450 and b5 oxidases
Mitochondrion
• P-450 and b5 oxidases
• Respiratory chain oxidation
Plasma membrane
• NADPH oxidase
Cytosol
• Xanthine oxidase
• Transition metals (Cu, Fe)
Peroxisomes
• Multiple oxidases
Lysosomes (in phagocytes)
• Myeloperoxidase
• NO synthase
Nucleus
O_2
O_2^-, H_2O_2, $OH^•$, NO
O_2^-, H_2O_2, $OH^•$, NO
O_2^-
Fe^{+2} Fenton Fe^{+3}
O_2^- SOD H_2O_2 $OH^•$ + OH^-
Catalase
H_2O
H_2O
Glutathione peroxidase
GSSG
2GSH
Glutathione reductase
Membrane lipid peroxidation
DNA fragmentation
Protein cross-linking and fragmentation
B. CELL INJURY BY FREE RADICALS
All membranes
• Vitamins E and A
• β-Carotene
Mitochondrion
• SOD
• Glutathione peroxidase
Peroxisomes
• Catalase
Cytosol
• SOD
• Vitamin C
• Glutathione peroxidase
• Ferritin
• Ceruloplasmin
C. NEUTRALIZATION OF FREE RADICALS—NO CELL INJURY

Figure 1-7

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
- 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

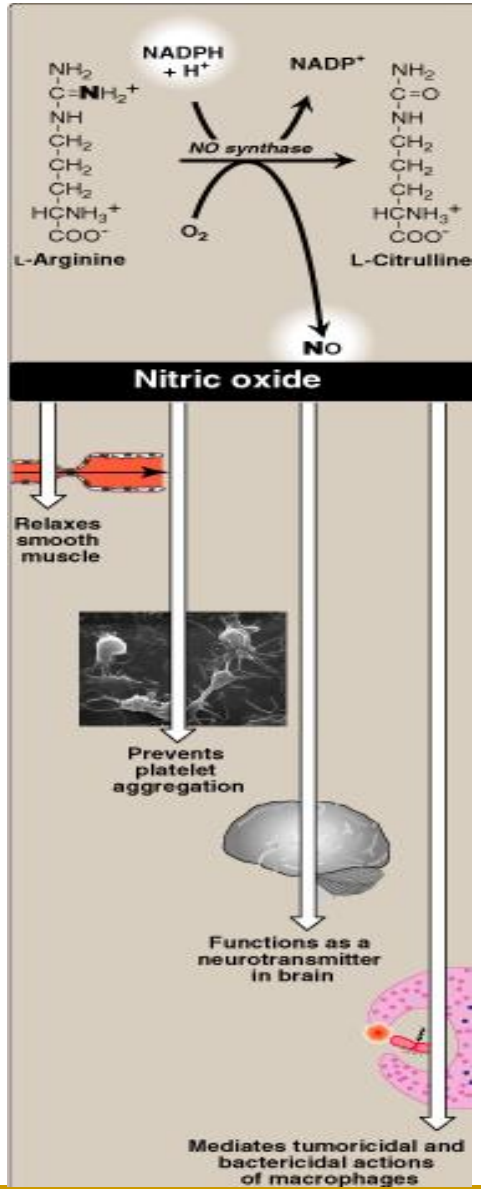
- The brain is highly susceptible to ROS-induced damage because of:
  - High concentrations of peroxidisable lipids
  - Low levels of protective antioxidants
  - High oxygen consumption
  - High levels of iron (acts as pro-oxidants under pathological conditions)
  - The occurrence of reactions involving 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 Ca^{2+} from intracellular stores)
  - **Cytoskeletal** damage
  - **Chemotaxis**
- Vascular effects:
  - Altered vascular tone and cerebral blood flow
  - Increased platelet aggregability
  - 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 **detrimental** (harmful) effects.
- Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes


NADPH + H+
NADP+
NO synthase
O2
L-Arginine
L-Citrulline
NO
Nitric oxide
Relaxes smooth muscle
Prevents platelet aggregation
Functions as a neurotransmitter in brain
Mediates tumoricidal and bactericidal actions of macrophages

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)

↑ **Lactic acid** in neurons → acidosis → promotes the pro-oxidant effect → ↑ the rate of conversion of $O_2^{\cdot -}$ to H_2O_2 or to hydroxyperoxyl radical

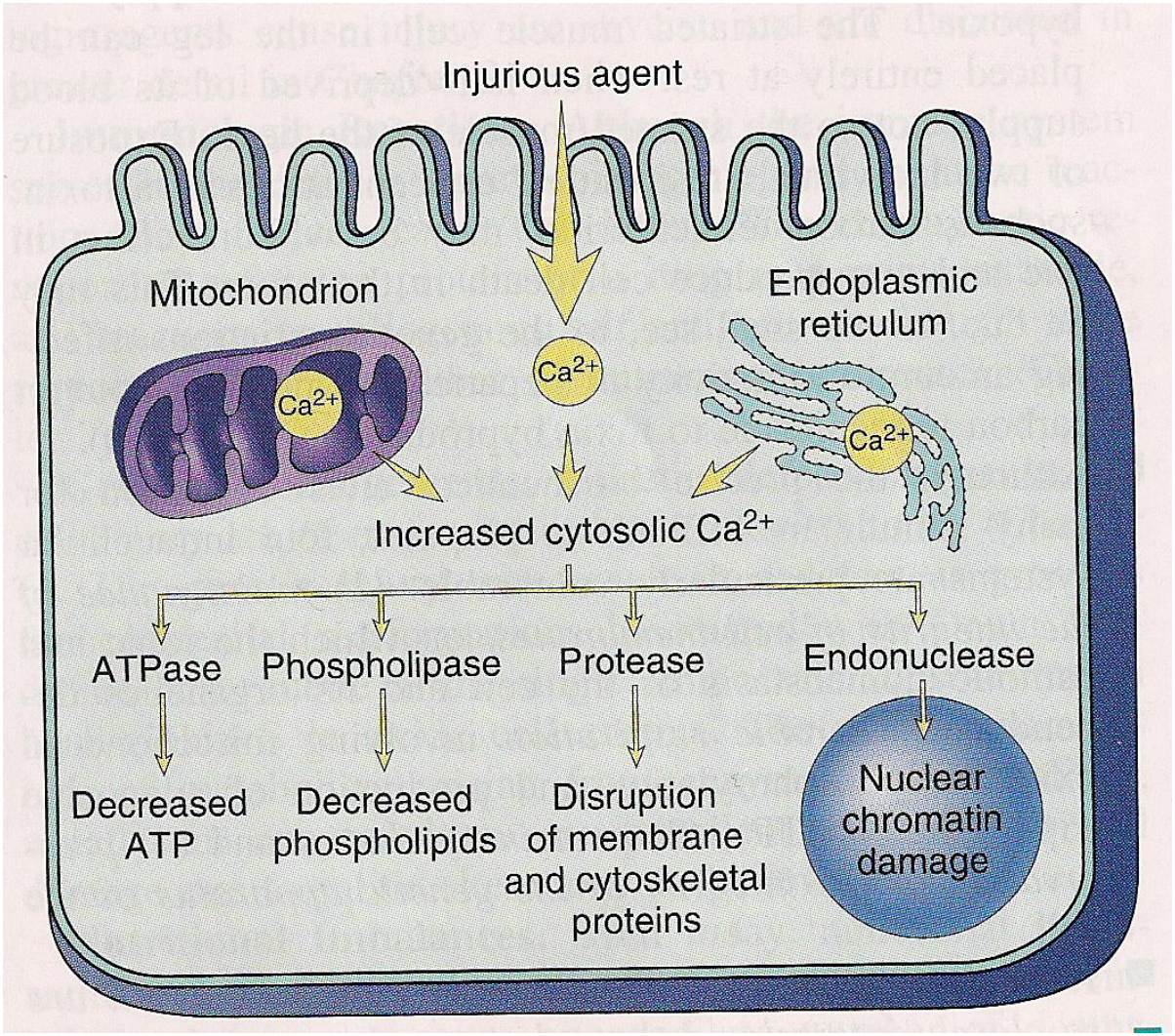
- Inhibition of ATP-dependent ion pumps
  - Membranes depolarization
  - Perturbance of transmembrane ion gradients

- **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



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

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

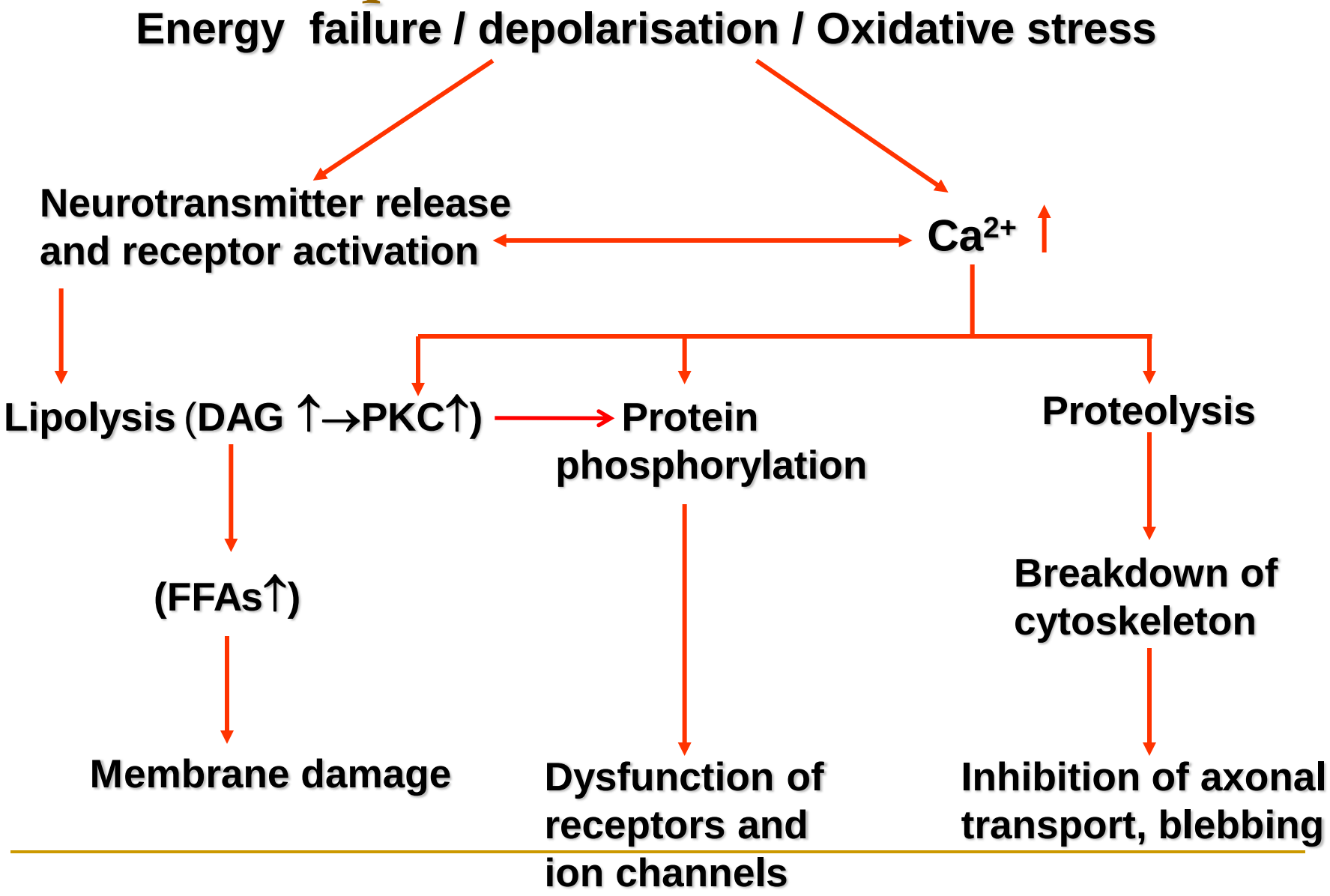
↓

Further depolarisation of cells

↓

Further calcium influx

Cosequences of brain ischemia



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

THANK YOU ☺