Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

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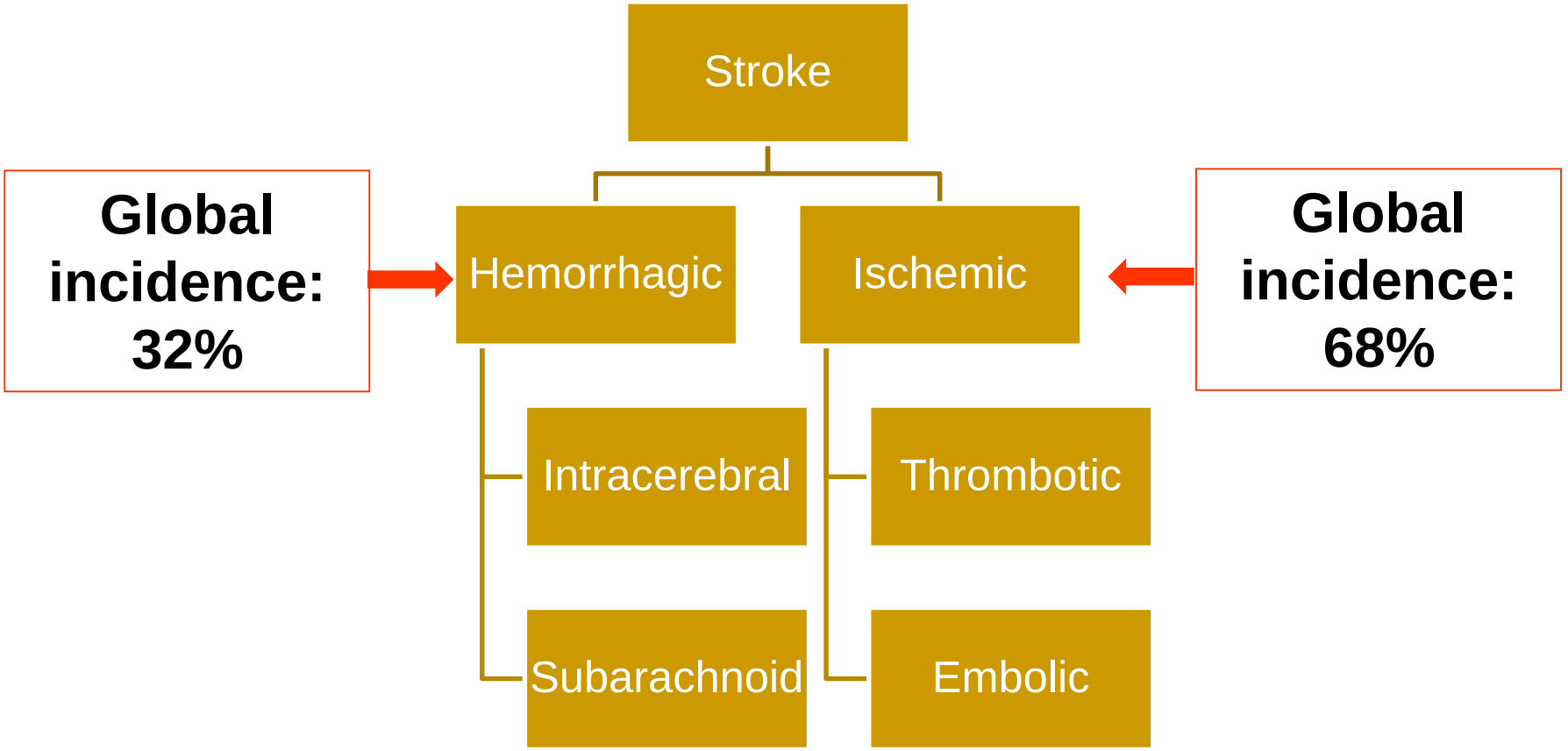
Objectives:

- 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

Cerebral Ischemia (Strokes) subtypes



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Characteristics of stroke subtypes

Stroke type	Clinical course	Risk factors	Other clues
Intracerebral hemorrhage	Gradual progression (min –hrs)	HTN, trauma, bleeding diatheses, illicit drugs, vascular malformations, certain races.	May be precipitated by physical activity. Patient may have reduced alertness.
Subarachnoid hemorrhage	Abrupt onset of sudden, severe headache.	Smoking, HTN, alcohol, genetic susceptibility (family history of subarachnoid hemorrhage) and sympathomimetic drugs	

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Characteristics of stroke subtypes, *continued..*

Stroke type	Clinical course	Risk factors	Other clues
Ischemic (thrombotic)	Stuttering progression with periods of improvement. Lacunes develop over hours or at most a few days; large artery ischemia may evolve over longer periods.	Atherosclerotic risk factors (age, smoking, DM, etc.). Men affected more commonly than women. May have history of **TIA.**	May have neck bruit.
Ischemic (embolic)	Sudden onset with deficit maximal at onset. Clinical findings may improve quickly.	Atherosclerotic risk factors. Men affected more commonly than women. History of **heart disease**	Can be precipitated by getting up at night to urinate, or sudden coughing or sneezing.

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Epidemiology of Stroke

- Globally:
  - Stroke is the second most common cause of mortality
  - Stroke is the third most common cause of disability
  - The incidence of stroke is:
    - decreasing in high-income countries
    - increasing in low-income countries
  - The overall rate of stroke-related mortality is decreasing in high and low income countries
  - The absolute number of people with stroke, stroke survivors, stroke-related deaths, and the global burden of stroke-related disability is high and increasing
  - Men have a higher incidence of stroke than women at younger but not older ages
  - Stroke incidence is higher in women ≥75 year old compared to men

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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

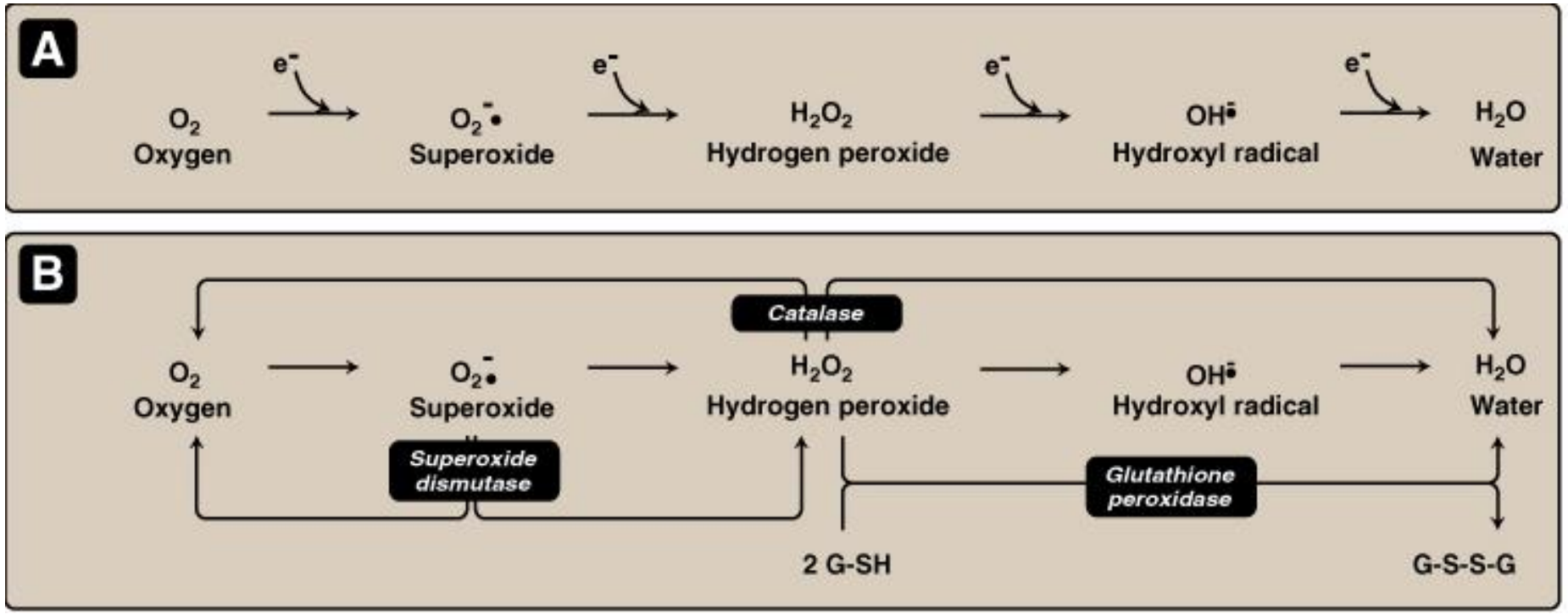
- Oxidative stress
- Metabolic stress
- Neurochemical response

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

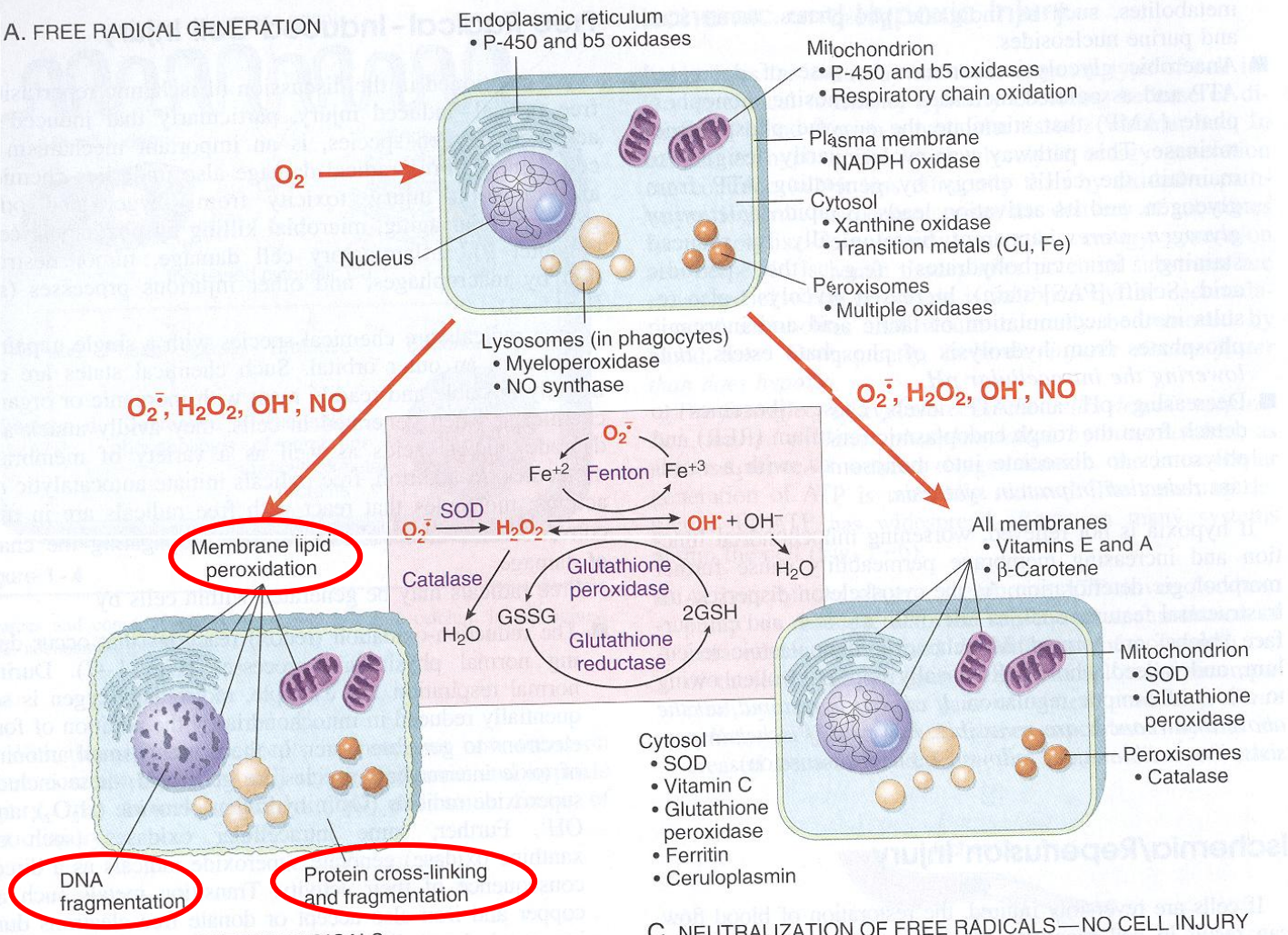

A. FREE RADICAL GENERATION
Endoplasmic reticulum
• P-450 and b5 oxidases
Mitochondrion
• P-450 and b5 oxidases
• Respiratory chain oxidation
Plasma membrane
• NADPH oxidase
Cytosol
• Xanthine oxidase
• Transition metals (Cu, Fe)
Peroxisomes
• Multiple oxidases
Lysosomes (in phagocytes)
• Myeloperoxidase
• NO synthase
Nucleus
O_2
$O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, NO
$O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, NO
$O_2^{\cdot-}$
Fe^{+2} Fenton Fe^{+3}
$O_2^{\cdot-}$ SOD H_2O_2 $OH^{\cdot}$ + OH^-
Catalase
H_2O
H_2O
GSSG
Glutathione peroxidase
Glutathione reductase
2GSH
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

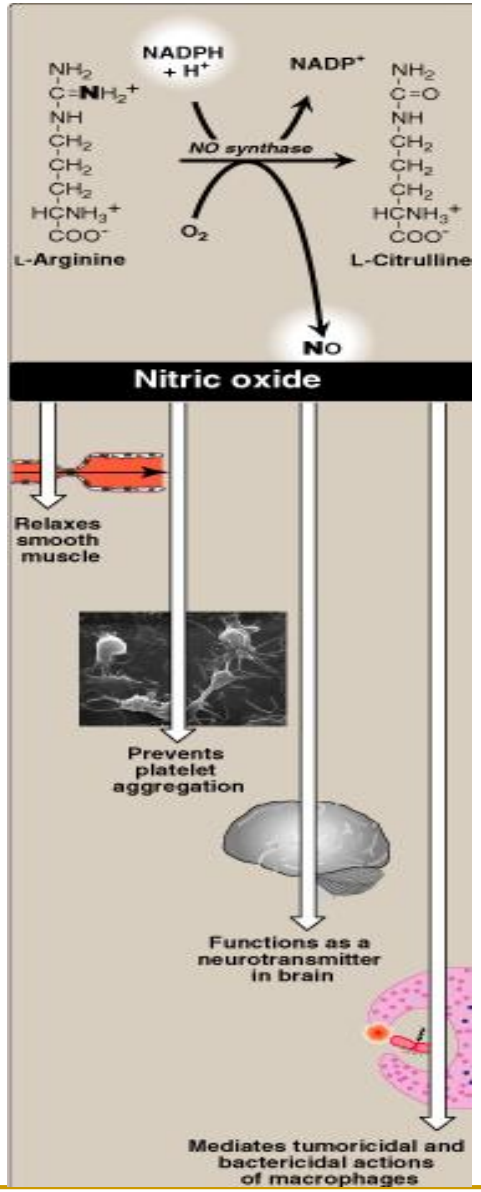
- The brain is highly susceptible to ROS-induced damage because of:
  - High concentrations of peroxidisable lipids
  - Low levels of protective antioxidants
  - High oxygen consumption
  - High levels of iron (acts as pro-oxidants under pathological conditions)
  - The occurrence of reactions involving 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

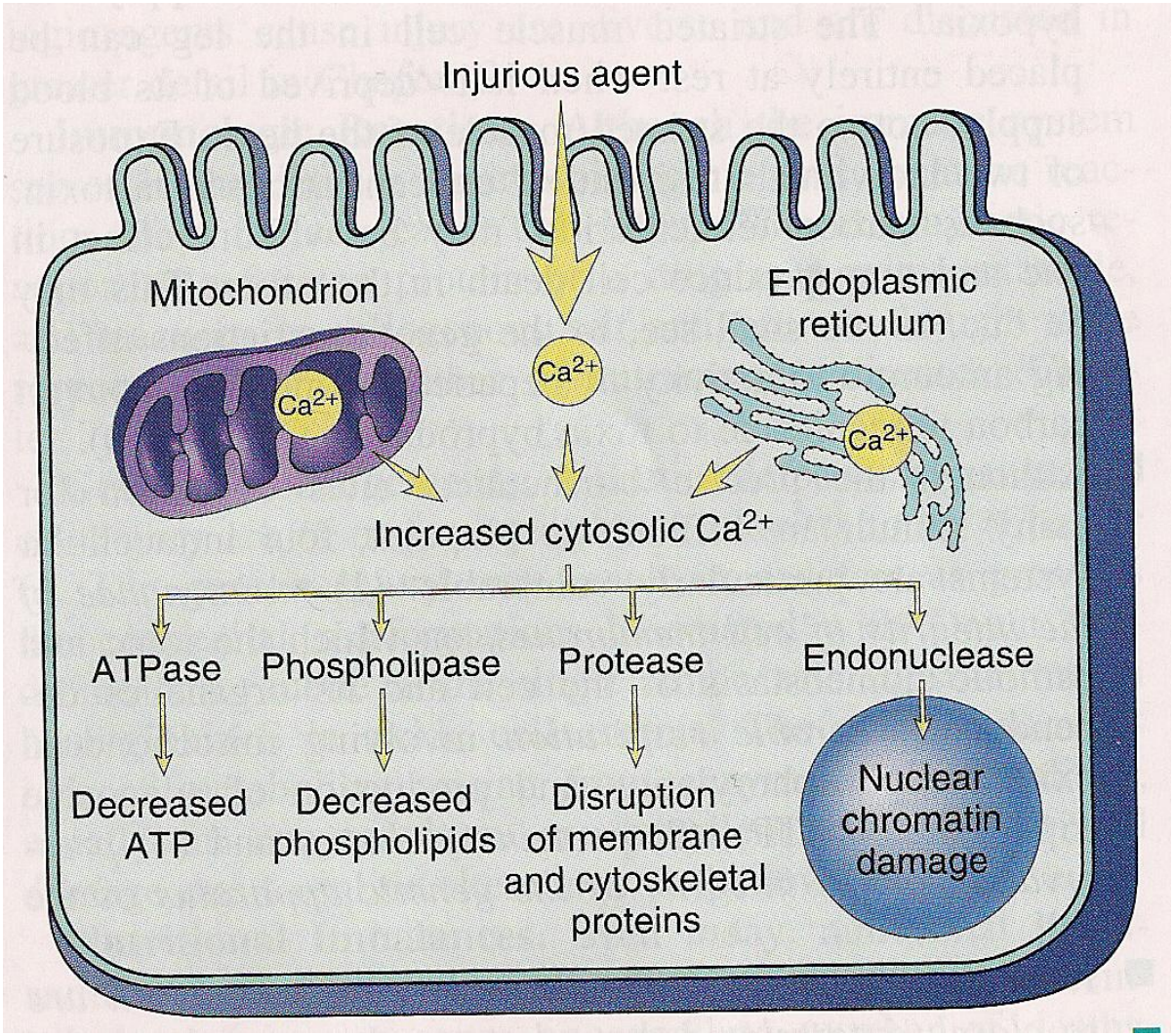
- Inhibition of ATP-dependent ion pumps
  - Membranes depolarization
  - Perturbance of transmembrane ion gradients

- **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

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

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

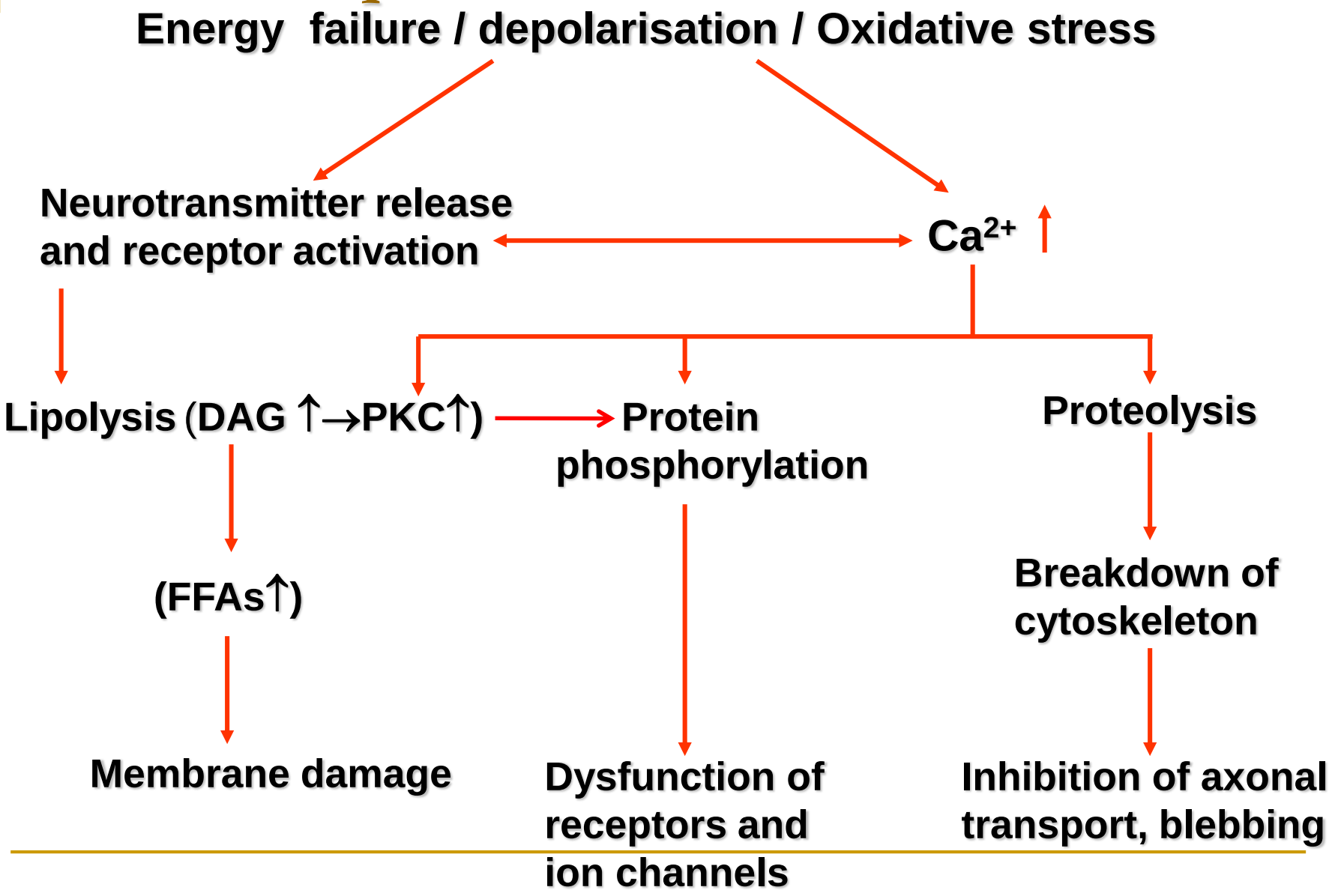
↓

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 ☺