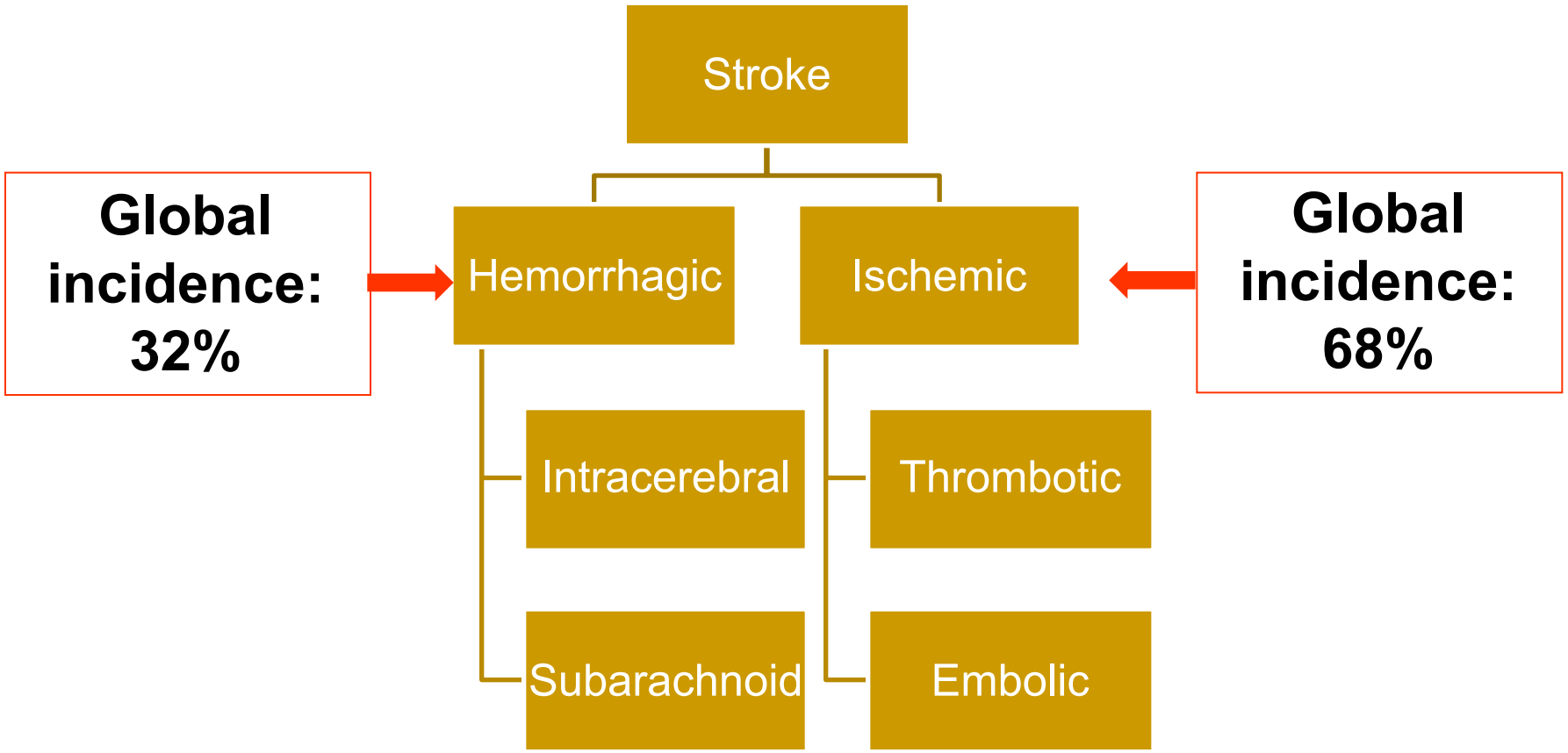
Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

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

Cerebral Ischemia (Strokes) subtypes



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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

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 and Regulatory and signaling proteins

Biochemical Responses to Ischemic Brain Injury

Oxidative stress

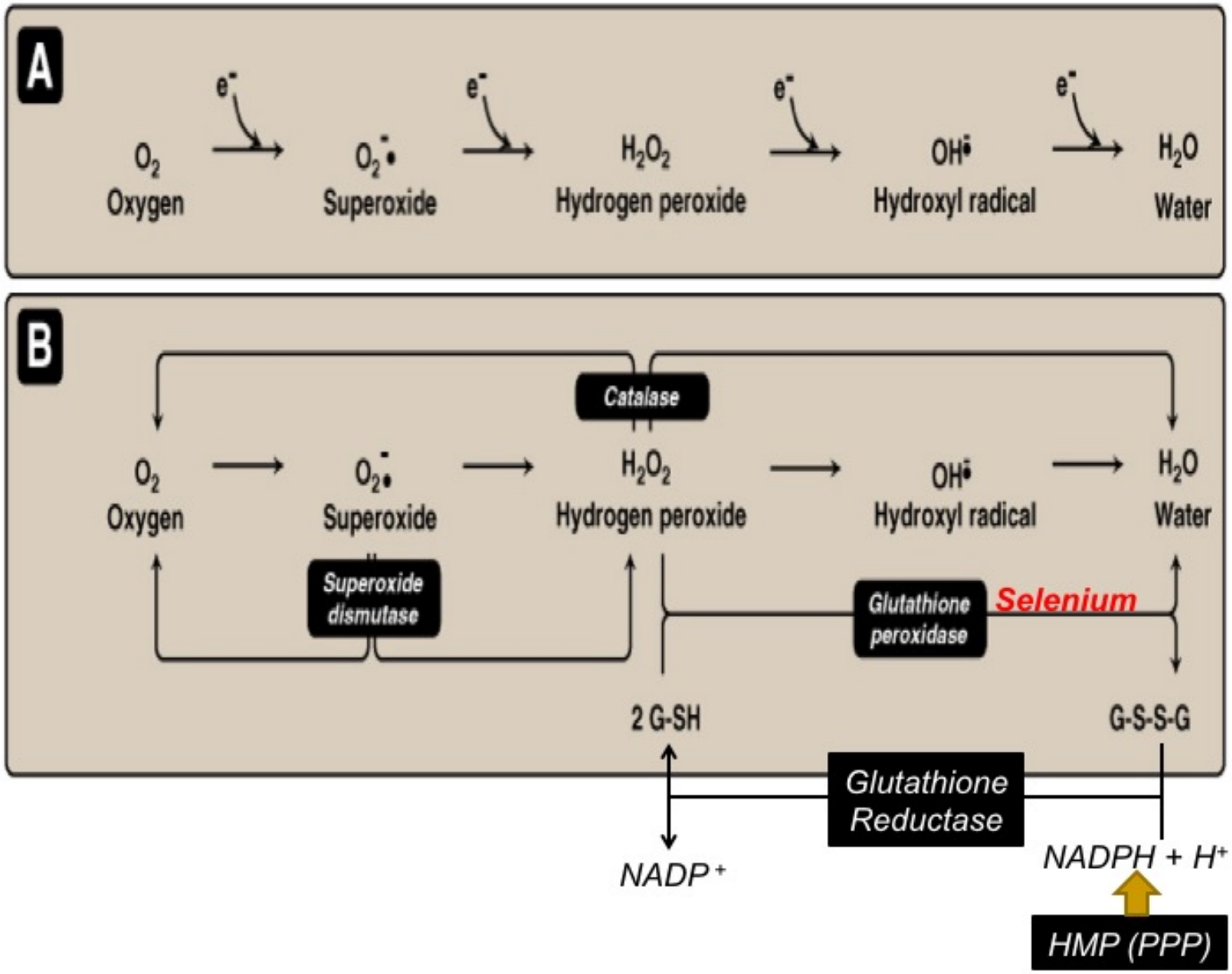
Metabolic stress

Neurochemical response

Oxidative stress

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

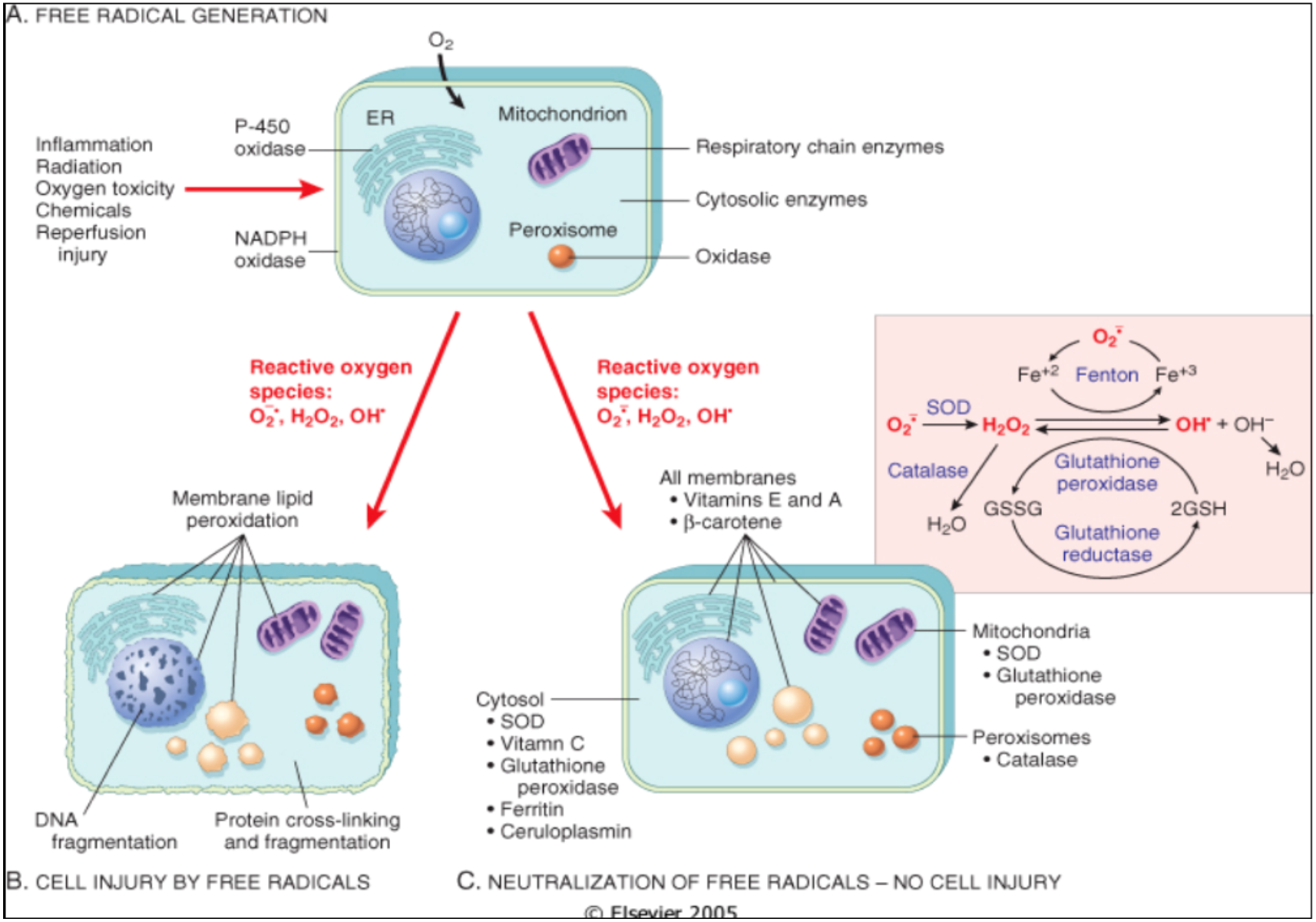
- They are mainly generated by microglia & astrocytes
- They modulate synaptic transmission & non-synaptic communication between neurons & glia
- During periods of increased neuronal activity, ROS & RNS diffuse to the myelin sheath of oligodendrocytes activating Protein kinase C (**PKC**) → posttranslational 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


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
Selenium
2 G-SH
G-S-S-G
Glutathione Reductase
NADP+
NADPH + H+
HMP (PPP)

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)


A. FREE RADICAL GENERATION
O_2
ER
Mitochondrion
P-450 oxidase
Inflammation
Radiation
Oxygen toxicity
Chemicals
Reperfusion injury
Respiratory chain enzymes
Cytosolic enzymes
Peroxisome
NADPH oxidase
Oxidase
Reactive oxygen species:
$O_2^{-\cdot}$, H_2O_2, $OH^{\cdot}$
Reactive oxygen species:
$O_2^{-\cdot}$, H_2O_2, $OH^{\cdot}$
$O_2^{-\cdot}$
Fe^{+2} Fenton Fe^{+3}
SOD
$O_2^{-\cdot}$ → H_2O_2 ⇄ $OH^{\cdot}$ + OH^-
H_2O
Catalase
Glutathione peroxidase
GSSG
2GSH
H_2O
Glutathione reductase
Membrane lipid peroxidation
All membranes
• Vitamins E and A
• β-carotene
Mitochondria
• SOD
• Glutathione peroxidase
Peroxisomes
• Catalase
Cytosol
• SOD
• Vitamn C
• Glutathione peroxidase
• Ferritin
• Ceruloplasmin
DNA fragmentation
Protein cross-linking and fragmentation
B. CELL INJURY BY FREE RADICALS
C. NEUTRALIZATION OF FREE RADICALS – NO CELL INJURY
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The brain and Oxidative stress

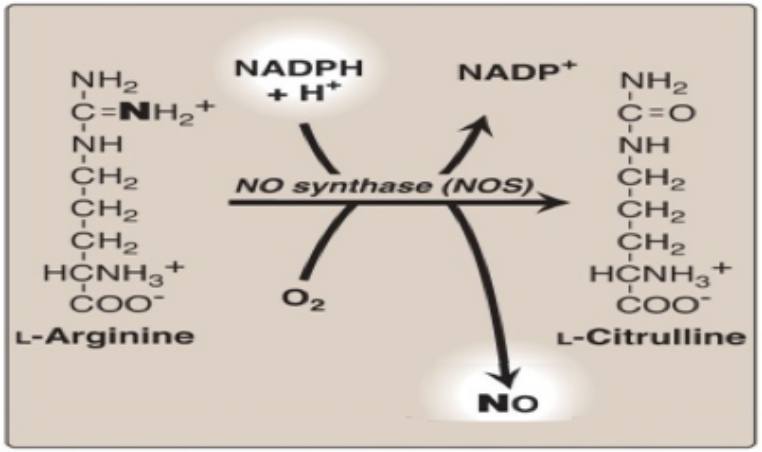
- The brain is highly susceptible to ROS-induced damage because of:
  - High concentrations of peroxidisable lipids
  - Low levels of protective antioxidants
  - High oxygen consumption
  - High levels of iron (acts as pro-oxidants under pathological conditions)
  - The occurrence of reactions involving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

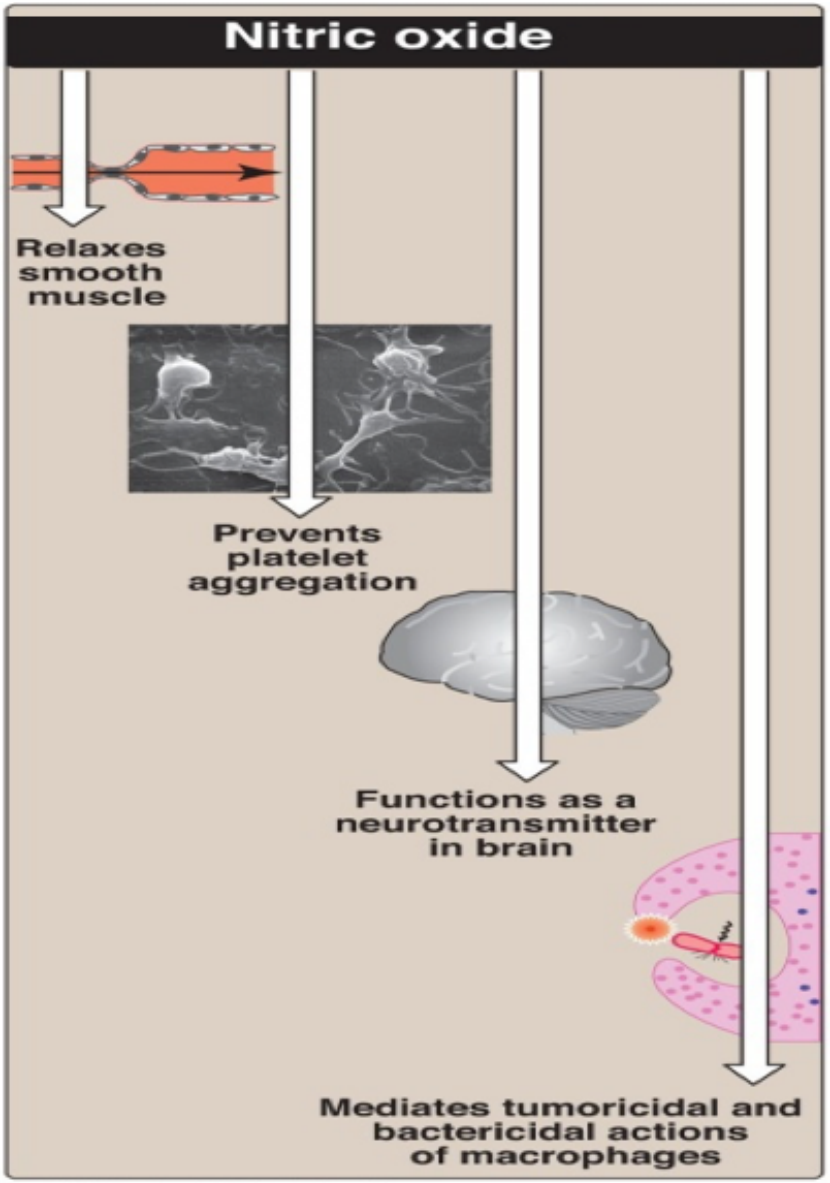
Molecular & Vascular effects of ROS in ischemic stroke

- **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 (NOS)
O2
NO
L-Arginine
L-Citrulline


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)

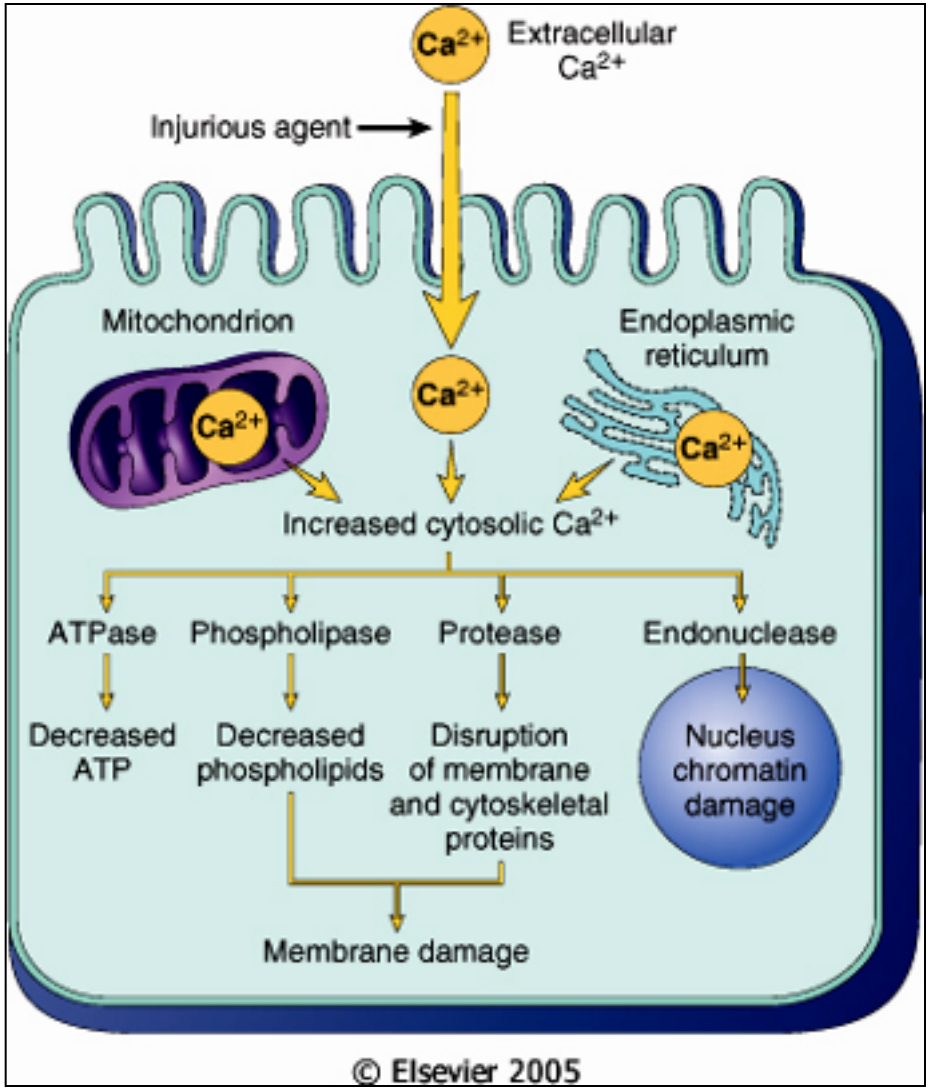
↑ **Lactic acid** in neurons → acidosis → promotes the pro-oxidant effect → ↑ the rate of conversion of $O_2^{\cdot -}$ to H_2O_2 or to hydroxyperoxyl radical

- 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

http://www.uptodate.com/contents/overview-of-the-evaluation-of-stroke

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

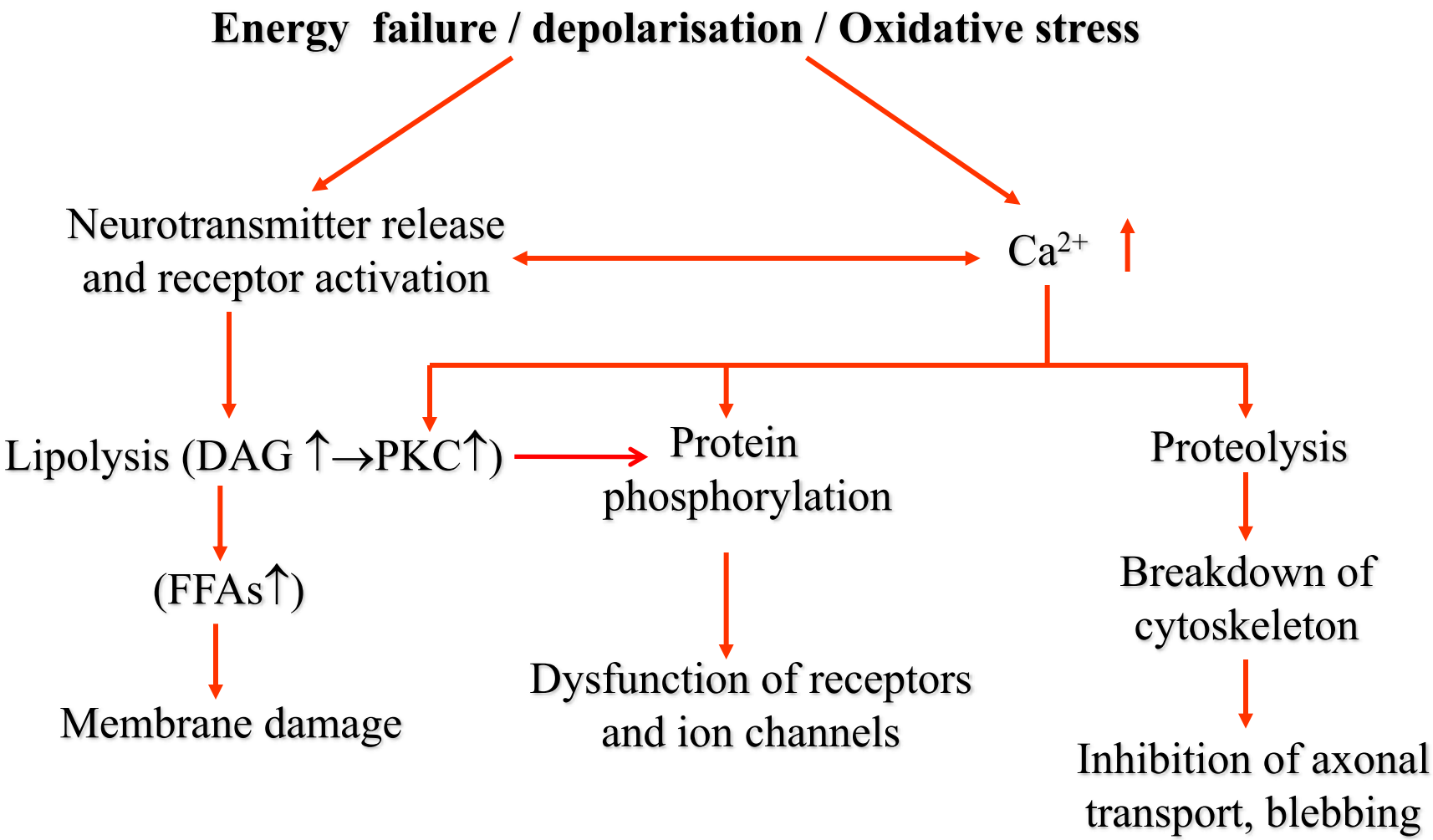
↓

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

References

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