Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

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

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Cell death mechanisms in cerebral ischemia:

Necrosis:

Commonly observed early after severe ischemic insults

Leading to

Apoptosis:

occurs with more mild insults and with longer survival periods

calcium-induced calpain-mediated proteolysis of brain tissue*

Substrates for calpain include:

- ✓ Cytoskeletal proteins
- ✓ Membrane proteins
- ✓ Regulatory and signaling proteins

*Explanation:

Calpains are cysteine proteases (perform proteolysis) whose enzymatic activities are strictly controlled by Ca^{2+}.
They have a physiological role, however when Ca^{2+} levels are high, calpains participate in cell death.

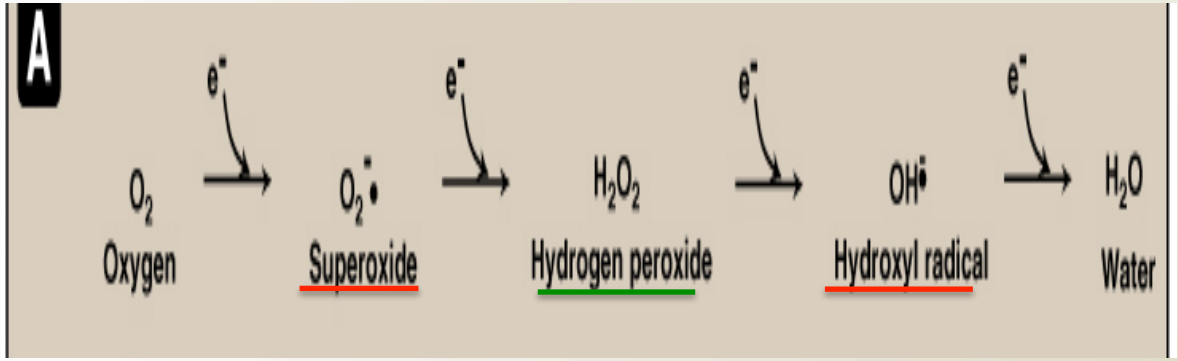
Biochemical Responses to Ischemic Brain Injury

1. Oxidative stress
2. Metabolic stress
3. Neurochemical response

1 Oxidative stress

Reactive Oxygen Species (ROS)

Formed as by-product of aerobic metabolism, reactions with drugs and environmental toxins or decreased antioxidant levels create oxidative stress



Oxygen-derived free radicals:

e.g., Superoxide and hydroxyl radicals

Non-free radical

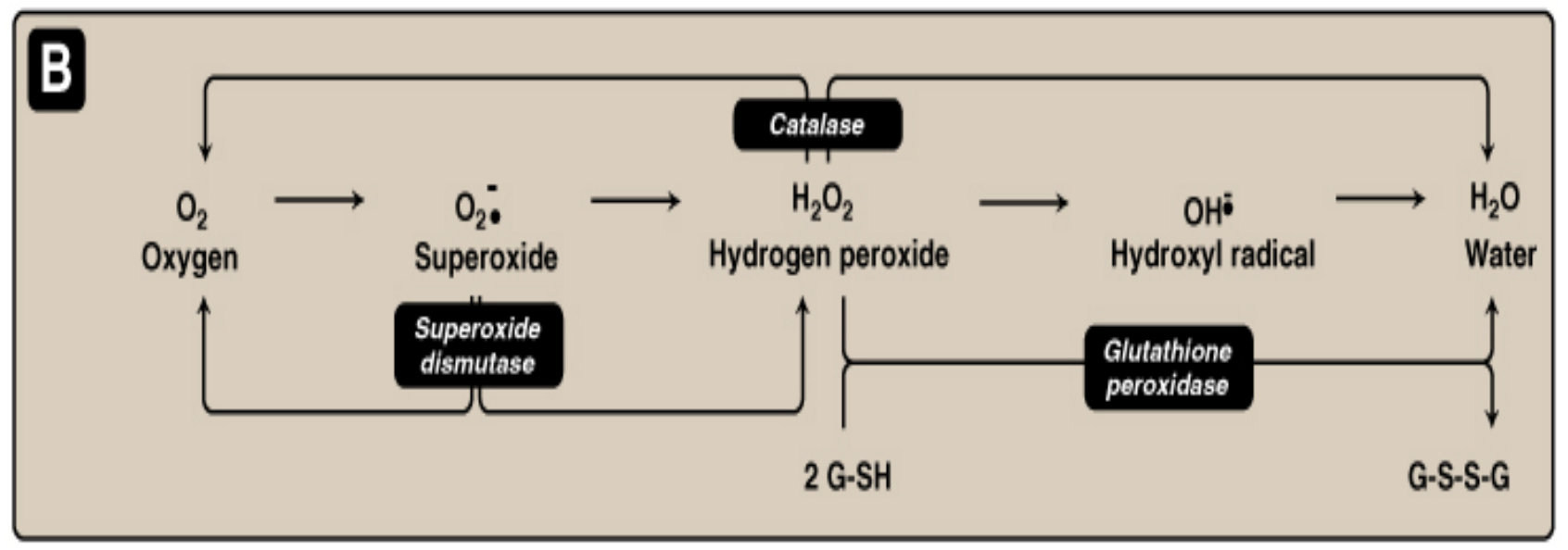
Hydrogen peroxide

A condition in which cells are subjected to excessive levels of Reactive Species (Oxygen or nitrative species) and 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, stroke and neurodegenerative diseases)

*In other words: Imbalance between oxidant production and antioxidant mechanisms

Antioxidant Mechanisms



Enzymes:

- Superoxide dismutase
- Catalase
- Glutathione system includes: (glutathione, NADPH, reductase, peroxidase & selenium)

These enzymes serve as a defense system to guard against the toxic effect of ROS.

They regulate neuronal signaling in both CNS & PNS

They are required for essential processes as learning and memory formation

Modulate synaptic transmission and non-synaptic communication between neurons & glia

Physiological roles of ROS and RNS[1] in the brain

In case of increased neuronal activity

They diffuse to the myelin sheath of oligodendrocytes

Activate protein kinase C (PKC)

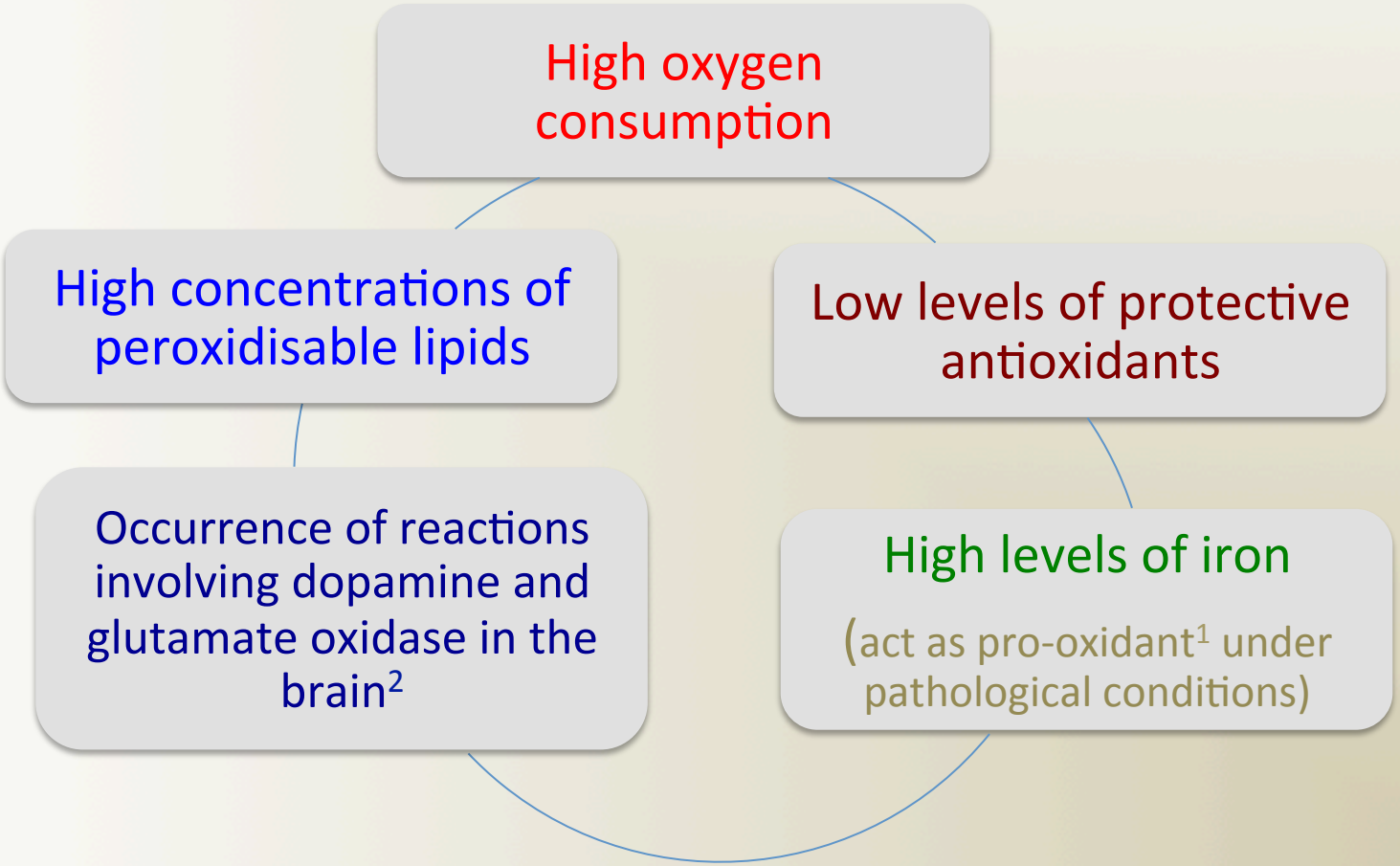
Post-translational modification[2] of myelin basic protein (MBP) by phosphorylation

Note:

ROS and RNS are commonly (normally) generated by microglia and astrocytes.

1"RNS": Reactive nitrative species.
2: modification of proteins after translation e.g. phosphorylation or glycosylation

Why is the brain highly susceptible to ROS-induced damage?



1: Pro-oxidant: They produce oxidative stress either by producing ROS or inhibiting antioxidant
2: These two enzymes produce free radicals through the pathway of glutamate and dopamine synthesis

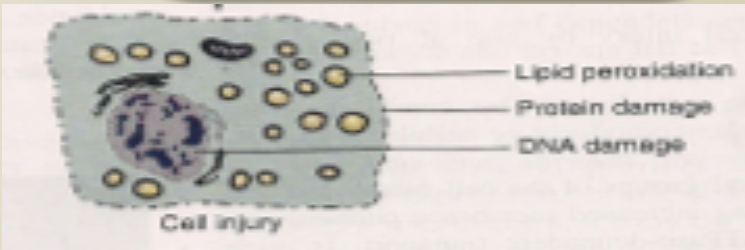
Effects of ROS in ischemic stroke:

Vascular effects:

- Altered vascular tone and cerebral blood flow
- Increased platelets aggregability.
- Increase endothelial cells permeability => Edema

Molecular effects:

- Lipid peroxidation[1]
- Protein denaturation
- Inactivation of enzymes
- DNA damage
- Cell signaling effects (release of intracellular Ca^{2+})
- Cytoskeletal damage
- Chemotaxis



1: Oxidative degradation of lipids, affect polyunsaturated fatty acids.

The role of Nitric Oxide (NO)[1] in the pathophysiology of cerebral ischemia

It may be beneficial or detrimental, depending upon **where and when NO is released**.

In case of Ischemia => Abnormal NO production

NO produced by:

Endothelial NOS (eNOS)	Neuronal NOS (nNOS)	Inducible NOS (iNOS)
Improve vascular dilatation and perfusion + Relaxation of smooth muscles.	**Detrimental effect.**	
Beneficial effect.		

Other actions of NO

- Prevent platelets aggregation
- Function as a neurotransmitter in the brain
- Modulate tumoricidal and bactericidal actions of macrophages

1: A highly diffusible stable gas synthesized from the amino acid Arginine by an enzyme called Nitric oxide synthase (NOS)

Metabolic stress

Biochemical changes in The brain during ischemia
(Ischemic cascade)

Ischemia

↓ Blood flow, O_2 and nutrients in cerebral arteries

Energy depletion (No ATP & creatine phosphate)

Inhibition of ATP-defendant ion pumps
(Membrane depolarization)

- Ca^{2+} Influx (translocation from extracellular to intracellular spaces)
- Na+ influx
- K+ efflux (K+-induced release of excitatory amino acids)

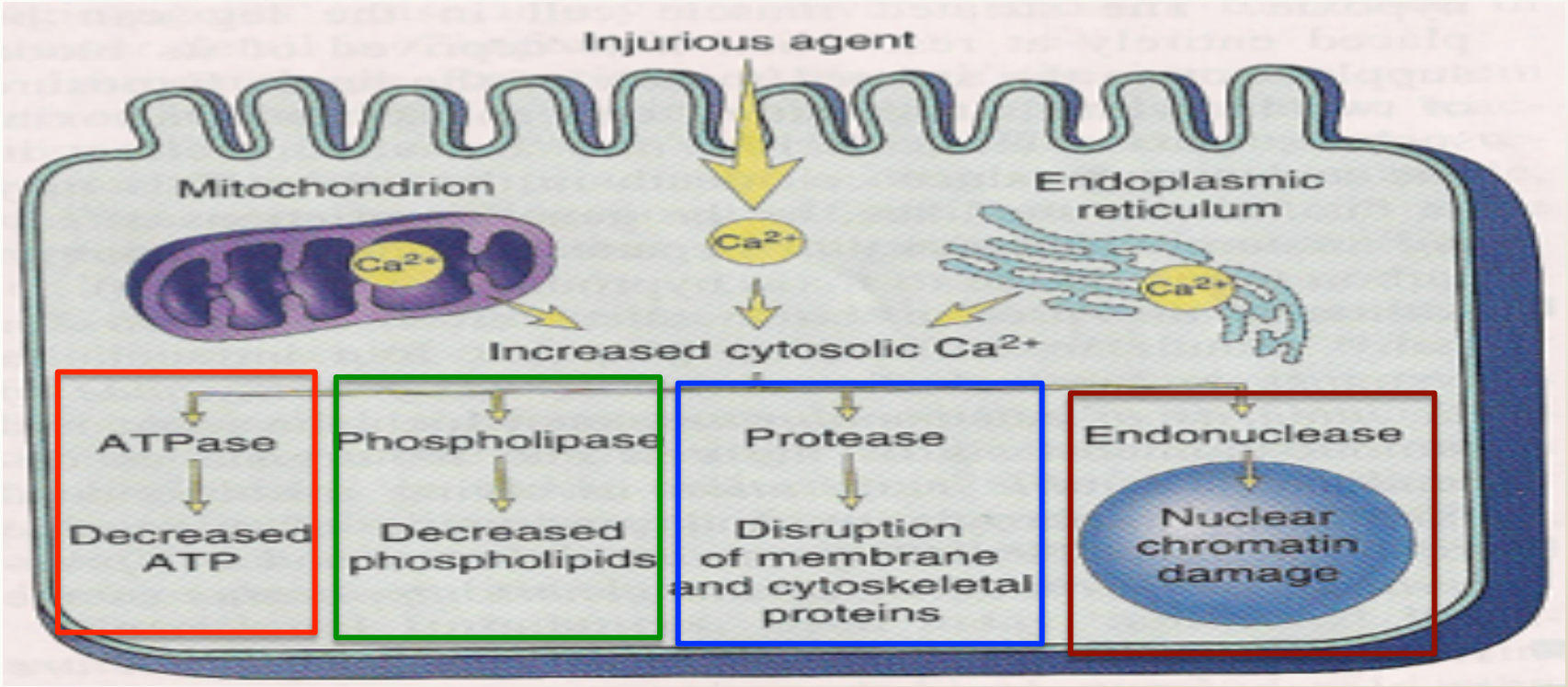
↑ Lactic acid in neurons
(because they turn to anaerobic glycolysis)

Acidosis

Promote pro-oxidant effect

↑ Rate of O_2 conversion into free radicals

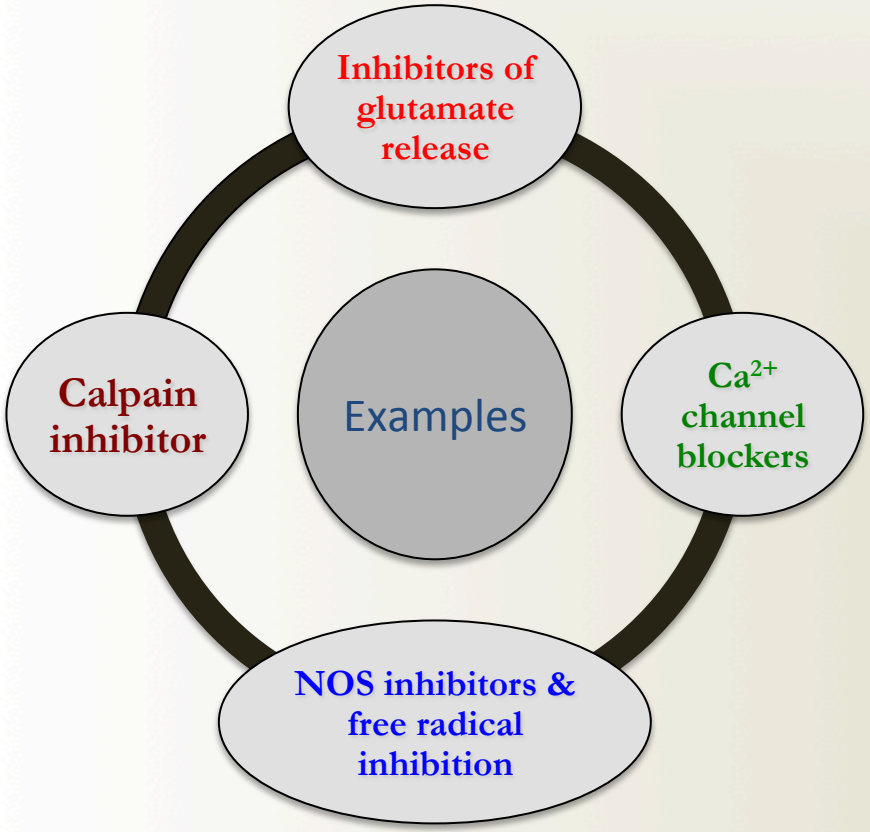
What are the consequences of Increased intracellular Ca^{2+} ?



Ca^{2+} influx will lead to activation of several enzymes which have detrimental effect such as:

1- ATPase => Degradation of ATP => Lead to further decrease of ATP.
2- Phospholipase => Lipolysis => Membrane damage.
3- Proteases (e.g. Calpain) => proteolysis => Breakdown of cytoskeleton
4- Endonuclease => DNA cleavage => DNA fragmentation .

Biochemical basis of pharmacological intervention



3 Neurochemical response

Following cerebral ischemia, extracellular levels of various neurotransmitters are increased:

- **Glutamate**
- **Glycine**
- **GABA**
- **Dopamine**

1- Which of the following cell death mechanism happen early after severe ischemic insults ?

A- Apoptosis
B- Necrosis
C- Dystrophic calcification
D- None of them

2- Which ONE of the following is NOT a molecular effect of ROS:

A- DNA damage.
B- Lipid peroxidation
C- Activation of enzymes
D- Chemotaxis

3- Oxidative stress has been implicated in which ONE of the following diseases:

A- Parkinson disease
B- Diabetes mellitus
C- Vitamin D deficiency
D- Night Blindness.

4- All the following statements are true EXCEPT:

A- High Oxygen consumption of the brain makes it more susceptible to ROS-induced damage.
B- The brain has low levels of Pro-oxidant as a protective property.
C- ROS and RNS are generated normally by microglia and astrocytes.
D- ROS and RNS are required for essential processes as learning and memory formation.

5- Which ONE of the following enzymes is involved in anti-oxidant mechanisms:

A- Calpain
B- Endonuclease
C- ATPase
D- Superoxide dismutase

6- Which ONE of the following events in the ischemic cascade will promote the pro-oxidant effect:

A- Ca efflux.
B- Alkalosis.
C- Turning of the neurons to anaerobic glycolysis which result in acidosis.
D- Increased extracellular levels of several neurotransmitters.

7- Which ONE of the following changes can be seen in the vasculature of a stroke patient induced by reactive oxygen species ?

A- Vasoconstriction
B- Hypocoagulability
C- Improved cerebral blood flow
D- Increased endothelial permeability

8- Brain biopsy of a stroke patient showed destruction of cytoskeleton proteins. This proteolysis is most likely mediated by:

A- Calpain .
B- Endonuclease.
C- ATPase
D- Phospholipase.

9- Protein phosphorylation in metabolic stress is due to increased levels of:

A- Intracellular K+
B- Extracellular Ca
C- Protein kinase A (PKA)
D- Protein kinase C (PKC)

10- Which ONE of the following forms of NOS is associated with inflammatory processes:

A- iNOS
B- eNOS
C- nNOS
D- All of them

ANSWERS:

1) B 2) C 3)A 4)B 5)D 6)C 7)D 8)A 9)D 10)A

If you have any questions or comments, don't hesitate to contact us

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Thank You!

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